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Neurobiological Disease Markers in Drug Naïve Schizophrenia Patients in Taiwan A Cross Sectional Study

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**Neurobiological Disease Markers in Drug Naïve
Schizophrenia Patients in Taiwan:
A Cross Sectional Study**

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A thesis submitted in fulfilment of the requirement for the degree of
Doctor of Philosophy in King's College London,
University of London
February 2014

Dedication

To my lovely family Mary, Bianca, and Alyssa

ABSTRACT

A major factor driving research into the pathophysiology of schizophrenia has been responsiveness to antipsychotic (dopamine antagonist) medication. Despite this it remains unclear whether there is a detectable disorder of dopamine function in patients who develop schizophrenia and if so, how it relates to other physiological markers of the condition. One reason why research in this area is such a challenge is the confounding effects of antipsychotic treatment.

I carried out a case control study on drug-naïve patients with schizophrenia who presented to the university hospital clinic in Tainan, Taiwan. I obtained a number of clinical and neurobiological measures, thought to be possible disease biomarkers. I assessed patients before they received treatment using the following methods: (I) single photon emission computed tomography (SPECT) - [^{99m}Tc] TRODAT-1 for dopamine transporter (DAT) availability; and (II) Electroencephalograms to test event-related potentials (ERPs). Such measures were also obtained on healthy controls and appropriate comparisons made. Clinical state was assessed using standard scales.

(I): DAT availability through [^{99m}Tc]-TRODAT-1 SPECT was compared between 47 drug naïve patients with recent onset schizophrenia and 112 healthy controls. I also conducted a random effects meta-analysis of the available literature synthesizing the results of six comparable published papers as well as my current data. The mean specific striatal binding ratio showed a trend for a reduction among the patients compared with controls (estimated difference = 0.071; 95% CI 20.01, 0.15; $P = .08$). There was an effect of sex, whereby females had a higher ratio of specific striatal binding than males. Age was negatively correlated with the ratio of specific striatal binding, both in patients and controls. The meta-analysis provided a pooled standardized effect size (Cohen's d) of -0.07 (95% CI: -0.31, 0.18; $p = 0.60$) for the patient versus control comparison in

TRODAT binding, with no evidence of heterogeneity between studies or publication bias. The findings suggest that striatal dopamine transporter levels are not altered in the early stages of schizophrenia before medication is introduced.

(II): ERP differences were explored between 36 drug naïve patients with schizophrenia and 138 healthy controls and P300 performance was examined in relation to dopamine transporter (DAT) availability. I also conducted a random-effects meta-analysis of the available literature synthesizing the results of 3 comparable published articles on drug naïve patients as well as my local study. The mean P300 ERP showed no overall significant difference between patients and controls in latency or in amplitude. However, there was an increase in P300 latency with age which was more pronounced in the patient group. No effects of DAT availability on P300 latency or amplitude were detected. The meta-analysis provided a latency pooled standardized effect size (Cohen's d) of -0.13 (95% CI -0.37, 0.12; $P = 0.31$), and an amplitude pooled standardized effect size (Cohen's d) of 0.48 (95% CI -0.002, 0.97; $P=0.05$) with patients showing a borderline significant reduction. My findings suggest the P300 ERP latency is not altered in the early stages of schizophrenia before medication is introduced, and the DAT availability does not influence the P300 ERP amplitude or latency. P300 ERP amplitude reduction could be an indicator of the progression of illness and chronicity.

The importance of taking into account medication effects is emphasized by this research as is the value of converging methodologies such as research with families and other 'at risk' populations and longitudinal cohort studies.

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I never had the thought to study in UK until few years ago my mentor who later became my local supervisor, Professor Yen Kuang Yang, consistently encouraged and supported me to make this academic journey a reality, that indeed turned my PhD study into a wonderful experience of my life.

First and foremost, I would like to show my appreciation to Professor Yen Kuang Yang, who was instrumental to my decision to embark on this PhD research. Furthermore, I would also like to express my gratitude for the overseas study support from the university where I work, National Cheng Kung University, and Taiwanese National Science Council which offered me a 3-year research grant.

I have been very lucky and appreciative to work with my PhD supervisors, Doctor Elvira Bramon and Professor Anthony David, who not only largely inspired my research mind but also led me to complete an excellent research work. Both of them have provided me the most inspirational mentorship and shown me the art of academic research.

I would like to express my sincere gratitude to my supervisors, Doctor Elvira Bramon and Professor Anthony David. Doctor Elvira Bramon has been very patient and encouraging, consistently giving sound advice when required. Professor Anthony David gave me the opportunity to do the PhD in the Institute of Psychiatry and was always generous in providing me with his inspiring insight and knowledge. My family and I still hold a wonderful memory of the Christmas evening we spent together at his house. Besides academic help, both Doctor Elvira Bramon and Professor Anthony David have been very helpful in making my stay in London comfortable.

Moreover, I would like to extend my special thanks to Professor Sabine Landau for her expert advice in statistics, and Doctor Oliver Howes for his expert advice in imaging studies.

I am very grateful for the help from my colleagues. Yen Kuang Yang, I Hui Lee, Tzung Lieh Yeh, and Po See Chen assisted me in recruiting and looking after the participants in my study. Ching Lin Chu, Tsai Hua Chang, and Yi Ting Tseng, supported me in administrative tasks such as participants' assessments and follow-ups. Chien Ting Lin offered his help in data analyses and management. Shih Hsien Lin also gave professional advice in data analyses. Furthermore, I would like to thank the patients who generously volunteered to participate in my study.

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STATEMENT OF CONTRIBUTIONS

The studies contained in my thesis are based on the baseline assessment within a cohort study of drug naïve patients with schizophrenia which is ongoing and funded by the Taiwanese Ministry of Science and Technology.

During the last 5 years my colleagues and I at the Department of Psychiatry, National Cheng Kung University (NCKU) Hospital and College of Medicine, NCKU in Tainan, Taiwan have been conducting a large cohort study of drug naïve patients with schizophrenia in the region of Tainan. I have been involved in the project from inception and have had a major role in the design and application

for ethical approval of this project. I have had a leadership role in the patient recruitment and data collection. I have been leading a research team including 5 psychiatrists and I have been responsible for coordinating my colleagues in the recruitment of eligible participants, and clinical assessments of patients before they received treatment. We also undertook comparable assessments on healthy controls for appropriate comparisons to be made. I designed the study protocol including the specific clinical assessments at baseline and follow up. I also performed the data management and all statistical analyses, and drafted the two manuscripts included in this thesis. The data presented in my thesis is drawn from the baseline assessment of my cohort study exclusively. The follow up of the cohort is now in its 5th year and I remain responsible for the coordination of all follow-up clinical assessments. I was also involved in designing the SPECT protocols.

I wrote chapter 1 (introduction) as well as chapter 4 (discussion) and all appendices with supervision from Prof. Yang and from my two PhD supervisors, Dr. Bramon and Prof. David, in London. In Chapters 2 and 3, I designed the study protocol. I also led and contributed to the recruitment of participants and all clinical assessments. I undertook all data management and quality control. I did all statistical analyses, including the meta-analyses myself with supervision from colleagues and supervisors. I drafted both manuscripts and thesis chapters with advice and comments from all my co-authors and supervisors.

My NCKU psychiatrist colleagues Drs. Yen Kung Yang, I Hui Lee, Tzung Lieh Yeh, Po See Chen, Ru Ben Lu contributed to the recruitment and clinical assessments of participants. Dr. Nan Tsing Chou (NCKU imaging expert) undertook the region of interest (ROI) measurements of SPECT and MRI, and provided me with imaging technical advice. Dr. I Hui Lee (NCKU expert in neurophysiology) designed and undertook the EEG experiments. She also did the signal processing of P300 data. However, I was responsible for all statistical analyses of the P300 data thereafter with input from my supervisor Dr. Bramon.

Dr. Oliver Howes (neuroimaging expert, KCL) provided advice and comments to both papers. Prof. Sabine Landau (professor of statistics at KCL)

provided me with statistical advice throughout the thesis and paper drafting. Prof. Yen Kung Yang (professor of psychiatry at NCKU) has been my mentor over the years and the head of department; he has been contributing ongoing advice and expertise throughout the project.

Finally, Dr. Elvira Bramon and Prof. Anthony David were my PhD supervisors and helped me throughout the data analyses, manuscript and thesis writing.

TABLE OF CONTENTS

Abstract	3
Acknowledgements	5
Chapter 1: Introduction	11
1.1. Brain Dopamine Imaging using [^{99m} Tc]-TRODAT-1 and other tracers	
1.2. Literature Review of the P300 Wave in Schizophrenia Research – The P300 as a Neurobiological Marker in Schizophrenia	
1.3. Aims and Hypotheses	
Chapter 2: Striatal dopamine transporter availability in drug naïve patients with schizophrenia: a case-control SPECT study with [^{99m}Tc]-TRODAT-1 and a meta-analysis	35
Introduction	
Methods	
Results	
Discussion	
Chapter 3: P300 waveform and dopamine transporter availability: a controlled EEG and SPECT study in medication naïve patients with schizophrenia and a meta-analysis	44
Introduction	
Method	
Results	
Discussion	

Chapter 4: Overall discussion and future directions	56
4.1. Summary of Thesis Findings	
4.2. DAT Availability: Methodological Considerations	
4.3. Interpretation of Thesis Findings	
4.4. Alternative Explanations for our Negative Findings	
4.5. Conclusions and Future Directions	
References	
Publications related to this thesis	87
Appendix 1: Further Demographic Data	88
Appendix 2: The Influence of Age on Receptor Binding and P300 and a Comparison between the Two	93
Appendix 3: Methodological Considerations – Inclusion of Correct Trials in the Averaging of EEG Signals	101
Appendix 4: The Demographic Matching of Patients and Controls in this study	104
Appendix 5: A Re-Analysis of Symptom Clusters Using A Five-Factor Model of the PANSS in My Patient Sample	108

Chapter 1

Introduction

Schizophrenia is a debilitating and often chronic disorder affecting about 1% of the population and with a typical onset in early adulthood (Bogren *et al.*, 2009; Simeone *et al.*, 2015). Current research indicates that the longer it remains untreated the worse the prognosis and functional outcomes. The identification and treatment of psychosis is vital as it is associated with increased risk of self-harm and suicide, significant reductions in life expectancy and social exclusion (Chang *et al.*, 2011; Dutta *et al.*, 2012; Lopez-Morinigo *et al.*, 2014; Osborn *et al.*, 2015). Antipsychotic medication remains the cornerstone of treatment in schizophrenia for both the acute presentation as well as the stabilisation-maintenance phase (Saunders *et al.*, 2011; Giannopoulos *et al.*, 2014). Despite the widespread availability of effective treatment for schizophrenia, its treatment outcome is highly variable. Whether neurobiological markers are useful alongside clinical measures in predicting disease outcome is still unknown.

A major factor driving research into the pathophysiology of schizophrenia has been the responsiveness to antipsychotic (dopamine antagonist) medication. However, besides its clinical benefits, antipsychotic exposure may precipitate progressive neuroanatomical brain changes and also adversely influence neural activity in schizophrenia (Lui *et al.*, 2010; Fusar-Poli *et al.*, 2013b). Furthermore, brain structural change could also be related to antipsychotic responsiveness independently of symptom severity and duration of illness (Hutcheson *et al.*,

2013). Despite this it remains unclear whether there is a detectable disorder of dopamine (DA) functions in patients who develop the disorder, and if so how it relates to other physiological markers of the condition. One reason why research in this area is such a challenge is the confounding effects of antipsychotic treatment.

A review of dopamine function in schizophrenia

Central dopaminergic hyperactivity continues to be one of the key hypotheses of the pathophysiology of schizophrenia (Seeman *et al.*, 1976; Howes & Kapur, 2009; Howes & Murray, 2014). Excess transmission at dopamine receptors and blockade of these receptors to treat psychosis were the primary focus in initial formulations back in the seventies (Matthysse, 1973; Snyder, 1976). Later in the 1990's, a modified dopamine hypothesis of schizophrenia was proposed by Davis *et al.* based on findings available at the time (Davis *et al.*, 1991). These included dopamine neuron lesions in the prefrontal cortex resulting in increased levels of dopamine and dopamine D₂ receptor density in the striatum (Pycock *et al.*, 1980). Further evidence came from applying dopamine agonists to prefrontal areas and how this reduced dopamine metabolite levels in the striatum (Scatton *et al.*, 1982). These studies suggested that the pathophysiology of schizophrenia was characterized by frontal hypodopaminergia resulting in striatal hyperdopaminergia.

Davis *et al.* further hypothesized that negative symptoms of schizophrenia resulted from frontal hypodopaminergia, and positive symptoms from striatal hyperdopaminergia. Furthermore, some studies also suggested that the attribution of salience to reward-predicting stimuli and the computation of prediction errors (Robinson & Berridge, 1993; Schultz *et al.*, 1993; Schultz *et al.*, 1997), are

indeed impaired in patients with schizophrenia due to functional activation potentially associated with striatal dopaminergic signaling, and this impairment may contribute to delusion formation (Heinz, 2002; Kapur, 2003).

As briefly outlined in the introduction of chapter 2, the dopamine hypothesis of schizophrenia has been revised as subsequent evidence has been emerging (Howes & Murray, 2014). According to Howes *et al.*, the dopamine hypothesis was built on the findings that antipsychotics work by blocking dopamine D_{2/3} receptors, and some drugs (such as amphetamine *etc.*) which activate the dopamine system can induce psychotic symptoms (Abi-Dargham, 2004; Curran *et al.*, 2004; Berman *et al.*, 2009; Howes *et al.*, 2009a). However, the alterations in D_{2/3} receptor availability of the dopamine system in schizophrenia are inconsistent and small at most, whilst there is no difference versus controls in transporter availability (Howes *et al.*, 2012; Chen *et al.*, 2013).

Moreover, evidence for elevated dopamine synthesis capacity, increased dopamine release and greater baseline synaptic dopamine levels in schizophrenia, have been found all with large effect sizes (Cohen's $d > 0.8$) (Howes *et al.*, 2012). Therefore, molecular imaging studies indicate presynaptic dysregulation as the major locus of dopamine dysfunction in schizophrenia (Howes *et al.*, 2009a; Lyon *et al.*, 2011; Howes *et al.*, 2012). Furthermore, the presynaptic dopamine abnormality is not simply a non-specific marker of psychiatric illness, since both dopamine synthesis capacity and dopamine release are not elevated in people with other common psychiatric disorders (Howes *et al.*, 2007), and it even has potential as a diagnostic test for schizophrenia (Bose *et al.*, 2008).

The onset of schizophrenia is frequently preceded by a prodromal phase of sub-clinical psychotic symptoms. People who present with “at risk” features have increased dopamine synthesis capacity on average (Howes *et al.*, 2009b; Egerton *et al.*, 2013), though not all “at risk” individuals are truly prodromal and most of them will never develop the disease. Elevated dopamine synthesis capacity is specific to those who proceed to develop frank psychosis. This greater dopamine synthesis capacity is associated with greater severity of sub-clinical symptoms only in those who proceed to develop clinical psychosis (Howes *et al.*, 2011). Studies using radiotracers selective for dopamine D_{2/3} receptors to index dopamine release following amphetamine use in patients with schizophrenia also indicate that greater dopamine release is associated with greater induction of psychotic symptoms, and dopamine release is greater in patients who are acutely psychotic than in stable remitted patients (Laruelle *et al.*, 1999). Acute depletion of intra-synaptic dopamine resulted in a larger increase in D_{2/3} receptor availability in patients with schizophrenia, and the increased occupancy of D_{2/3} receptors by dopamine occurred both in first-episode drug naive patients and in previously treated chronic patients experiencing an episode of illness exacerbation (Abi-Dargham *et al.*, 2000). In conclusion, a link between greater dopamine dysfunction and the development of more severe psychotic symptoms is indicated in patients with schizophrenia, and it suggests that the dopamine dysfunction is dynamic, increasing with the worsening of the disorder.

The importance of dopamine in cognition

Patients with schizophrenia show impaired cognitive functions which could be induced by an increased dopamine release that causes a catastrophic jump of

the dorsolateral prefrontal cortex activity from a low to a high level (Tanaka, 2006). This finding may account for the dorsolateral prefrontal cortex over-activation in patients with schizophrenia, and the cortical dopamine D_{2/3} receptor function may be more involved in some cognitive functions (i.e. attention, fluency and planning) in patients with schizophrenia than in healthy people according to a SPECT imaging study (Fagerlund *et al.*, 2013). Some studies report an inverse linear relationship between dopamine activity and word production in patients with schizophrenia who were tested prior to and following methylphenidate infusion during each phase, at the onset of their first-episode (acute phase) and then again after they were clinically stable (stabilization phase) (Szeszko *et al.*, 1999). Similarly, using PET, Slifstein *et al.* reported deficits in dopamine release capacity in the cortex of patients with schizophrenia (Slifstein *et al.*, 2015). Hence the literature shows that changes in brain dopamine levels could influence the impairments in cognitive functions described among patients with schizophrenia.

The relationship between dopamine and psychopathology in psychosis

Recent imaging studies have illuminated the relationship between dopamine activity in various parts of the brain and psychotic symptoms. According to an fMRI study in patients with schizophrenia, brain intrinsic activity is changed in the striatum, and this corresponds to disorder states and symptom dimensions. The intrinsic activity of the striatum is increased in the dorsal part and correlates with positive symptoms such as delusions and hallucinations during an acute episode of psychosis according to Sorg *et al.*, 2013. The activity of the ventral striatum however was found to be increased and correlated with negative symptoms, such as emotional withdrawal and blunted affect, in the same patients

when they were re-scanned whilst being in remission about 9 months later (Sorg *et al.*, 2013). Alterations in dopamine D₂ receptor function in the extra-striatal region may underlie the positive symptoms of schizophrenia (Suhara *et al.*, 2002; Kessler *et al.*, 2009). Furthermore, patients who had greater symptom reduction with antipsychotic treatment experienced more severe positive psychotic symptoms during the Amphetamine Challenge Test (ACT) (Pandurangi *et al.*, 1989), which reinforces the key role of dopamine in the genesis of psychotic symptoms.

Sex differences in dopamine neurotransmission

There is lower dopamine D₂ receptor affinity in females, which suggests an increased endogenous striatal dopamine concentration, that may contribute to the differential vulnerability of males and females to psychiatric disorders like schizophrenia (Pohjalainen *et al.*, 1998). Sex differences in striatal presynaptic dopamine synthesis have been also mentioned; thus healthy women were found to have significantly higher striatal ¹⁸F-DOPA uptake (Ki values) than healthy men, indicating females have a higher striatal presynaptic dopamine synthesis capacity than healthy males of similar age. Moreover, there was a negative correlation between striatal ¹⁸F-DOPA Ki values and age in men but not in women, indicating a relative decrease in dopamine activity with age in men but not in women (Laakso *et al.*, 2002). However, higher DAT availability was found in healthy females than in healthy males via single-photon emission tomography imaging with [¹²³I] FP-CIT (Lavalaye *et al.*, 2000). This might counteract the difference in dopamine synthesis and thus explain there is no increased risk in females. Finally, a similar sex effect was found in patients with schizophrenia

(Lavalaye *et al.*, 2001).

Further sex differences in dopamine function have been reported among patients with schizophrenia. For example, drug-free male patients showed a positive correlation of plasma homovanillic acid, a central dopamine metabolite, with negative symptoms while females showed no significant relationship with any psychotic symptoms (Zhang *et al.*, 2001a; Zhang *et al.*, 2001b). The symptoms of schizophrenia could be influenced by the dopamine D₂ receptor system, and modulated by estrogens since a negative correlation was found between symptoms and plasma estrogen levels (Hafner *et al.*, 1993). Indeed, it has been proposed that estrogen treatment might be beneficial for females with schizophrenia by way of a direct effect on dopamine and serotonin systems or via an indirect prolactin-mediated effect (Kulkarni *et al.*, 2001) .

Dopamine function and ageing

Dopamine D_{2/3} receptor and DAT availabilities in the striatum have been found to decline with age in healthy individuals (Volkow *et al.*, 1994; Volkow *et al.*, 1996a; Mozley *et al.*, 1999; Lavalaye *et al.*, 2000; Chen *et al.*, 2005). In healthy individuals DAT availability shows an estimated 6.6% decrease per decade of life (Volkow *et al.*, 1996a). Some in vivo investigations of ageing effects on D2 receptors also have consistently shown age-related decreases in striatal D2 receptors binding (Antonini & Leenders, 1993; Antonini *et al.*, 1993; Volkow *et al.*, 1996b). However, some investigators suggest that the effects of aging on DAT do not appear to be linear. Most effects on DAT availability seem to occur during young adulthood before people reach the age of 40. The

distribution of ageing effects then appears to remain relatively stable until late in life (Mozley *et al.*, 1999).

Older patients with schizophrenia have a narrower therapeutic window for antipsychotic drugs than do younger patients (Uchida *et al.*, 2014), partially due to an age related decrease in striatal dopamine D₂ binding sites (Antonini & Leenders, 1993; Antonini *et al.*, 1993; Ichise *et al.*, 1998). This decrease with age in some of the key elements of the dopaminergic system explains in part how patients become more sensitive to antipsychotics as they become older, and the need to lower antipsychotic doses for older patients with schizophrenia (Uchida *et al.*, 2009).

The effects of ageing on dopamine function are well recognized. In my results chapters I have accounted for the effects of ageing and discussed how they could influence my findings.

(1.1) Brain Dopamine Imaging using [^{99m}Tc]-TRODAT-1

Although it is difficult to measure dopamine levels directly in humans, neurochemical imaging techniques such as single photon emission computed tomography (SPECT) provide indirect indices of dopamine synthesis and release, and putative synaptic dopamine levels (Howes & Kapur, 2009). Synaptic dopamine competes with the radio-ligand and leads to a reduction in radiotracer binding and this provides an indirect index of released dopamine (Laruelle, 2000).

Dopamine transporters (DATs) are mainly located in the presynaptic membrane on the terminal of the dopaminergic projection and they regulate the dopamine level in the synaptic cleft (Nirenberg *et al.*, 1996). Hence DAT

dysfunction has been implicated in a number of psychiatric and neurological disorders (Bannon, 2005; Lindsey & Gatley, 2006).

A number of positron emission tomography (PET) and single photon emission computed tomography (SPECT) radio-ligands are available for DAT imaging in humans (Varrone & Halldin, 2010). Several ^{99m}Tc -labeled tropane analogues have been synthesized and been evaluated as DAT imaging agents (Kung *et al.*, 1996; Meltzer *et al.*, 1997; Booij *et al.*, 1999). Among these, ^{99m}Tc -labeled TRODAT-1 is a selective DAT SPECT imaging agent that binds specifically at the DAT site of dopamine neuron terminals in both healthy subjects and patients with neuropsychiatric disorders (Marek *et al.*, 1996; Staffen *et al.*, 2000; Huang *et al.*, 2001; Huang *et al.*, 2003; Huang *et al.*, 2004; Seibyl, 2008).

In this project, [^{99m}Tc]-TRODAT-1 was prepared from a pre-formulated lyophilized kit provided by the Institute of Nuclear Energy Research (Lung-Tan, Taiwan) (Wey *et al.*, 1998). The kit was reconstituted with 1,110 MBq (30 mCi) freshly eluted ^{99m}Tc -sodium pertechnetate in 5 mL normal saline solution and autoclaved at 121°C for 30 minutes to complete the labelling. After cooling to room temperature, [^{99m}Tc]-TRODAT-1 with a radiochemical purity of 90% (determined by a dual-strip instant thin-layer chromatography method) was obtained in a neutral solution (pH 7.0-7.5). The shelf life of the lyophilized kit is over 2 months when stored at room temperature (Liao *et al.*, 2001).

The demonstration of the reproducibility of [^{99m}Tc]-TRODAT-1 SPECT outcome measurements in humans is critical for clinical applications. A [^{99m}Tc]-TRODAT-1 SPECT study in patients with Parkinson's disease revealed a good test-retest reproducibility in striatum with a mean variability of 10.2% and an intra-class correlation coefficient (ICC) of 0.95 (Hwang *et al.*, 2004). Since the concentrations of DAT are age-dependent (Mozley *et al.*, 1999), it is critical to

establish the test-retest reliability of [^{99m}Tc]-TRODAT-1 SPECT for DAT concentrations measurement in normal young adults. Indeed, representative test-retest [^{99m}Tc]-TRODAT-1 brain SPECT images in young men showed good reproducibility between test and retest. The overall variability and ICC of test-retest specific uptake ratio (SUR) in caudate, putamen and striatum were 7.32-9.17% and 0.83-0.96, respectively (Yeh *et al.*, 2012).

Other DAT radiotracers

Although patterns of altered radiotracer uptake are similar among the different presynaptic dopaminergic imaging agents, the compounds can evaluate different aspects of cellular function and may be used in different ways (Table 1). The DAT agents bind to a protein transporter located on the presynaptic membrane, providing a measure of transporter density as an indirect measure of nerve terminal integrity.

According to Seibyl, dopaminergic radiotracers differ in important ways which dictate their application in clinical and research functions. The key differences include the nuclide for PET or SPECT, the pharmacokinetics of brain uptake and washout and their selectivity for target sites (Seibyl, 2008). The specific PET or SPECT radiotracer use depends on the clinical or research question to be addressed by imaging (Laruelle *et al.*, 1994; Seibyl *et al.*, 1997; Tsuchida *et al.*, 2004). Logistical factors such as radiotracer availability or the time to imaging post injection also guide the radiotracer selection.

SPECT tracers, including [^{123}I] beta-CIT (also known as RTI-55) (Shaya *et al.*, 1992; Seibyl *et al.*, 1997), [^{123}I] IPT (Kung *et al.*, 1995; Malison *et al.*, 1995), and [^{123}I] FP-beta-CIT (Neumeyer *et al.*, 1994; Kuikka *et al.*, 1995), have been

used in vivo for evaluation and assessment of dopaminergic neuronal function. Despite the success of these iodine-123-labeled tracers as SPECT imaging agents, a tropane analogue incorporating the radionuclide technetium-99m would be more desirable. ^{99m}Tc , as an isotope for SPECT imaging, is preferred to other nuclides due to greater photon flux per unit of radiation dose delivered to the patient. Furthermore, there are several characteristics which make this radionuclide very attractive: (1) ^{99m}Tc has a convenient 6-hour physical half-life, (2) it is inexpensive compared to ^{123}I , since ^{123}I is a cyclotron produced isotope (cost about \$30/mCi), a similar ^{99m}Tc labelled agent (^{99m}Tc cost about \$0.30/mCi) will be highly desirable for routine clinical studies and (3) it is available around the clock as a solution of ^{99m}Tc pertechnetate ($^{99m}\text{TcO}_4^-$) in normal saline from a commercially available $^{99}\text{Mo}/^{99m}\text{Tc}$ generator. The advantage of ready availability and ease of use with ^{99m}Tc agents provide a powerful incentive for their development (Kung *et al.*, 1997; Kung, 2001). One of the most attractive attributes of [^{99m}Tc]-TRODAT-1 as a DAT imaging agent is that it is prepared by a kit formulation (Choi *et al.*, 1999).

The Department of Health in Taiwan approved [^{99m}Tc]-TRODAT for clinical use in 2005. It remains relatively underutilized due to high costs and extensive bureaucratic requirements. However, the ready availability of ^{99m}Tc plus the convenient kit formulation makes this agent ideally suited as a routine clinical procedure for imaging the dopamine transporters in the brain. Hence I chose ^{99m}Tc -labeled TRODAT-1 as the DAT SPECT imaging agent for my project.

Table 1. Comparison of Presynaptic Markers of Dopaminergic Function commonly used in neuropsychiatric disorders

Radiotracer	¹⁸ F-DOPA	¹²³ I FP-CIT	¹¹ C-VMAT	¹²³ I β-CIT	^{99m} Tc-TRODAT	¹²³ I-Altropane
Molecular target	Dopamine metabolism	Dopamine transporter	Vesicular transporter	Dopamine transporter	Dopamine transporter	Dopamine transporter
Highest uptake	Striatum	Striatum	Striatum	Striatum	Striatum	Striatum
Target: background tissue ratio	Intermediate	High	Low	High	Low	Low
Optimal imaging time after injection	0 to 2 hours	3 to 4 hours	0 to 1.5 hours	8 to 24 hours	3 to 4 hours	0.25 to 0.75 hours
Comments	Has been used to directly evaluate substantia nigra	Commercial diagnostic use in Europe	New ¹⁸ F version in clinical trials	Used in large PD disease progression studies	High DAT:SERT selectivity	In commercial development in the US

Adapted and adjusted from: Seibyl, J. P. Single-photon emission computed tomography and positron emission tomography evaluations of patients with central motor disorders. Semin Nucl Med 2008

¹⁸F-DOPA: 6-fluoro-(18F)-L-3, 4-dihydroxyphenylalanine

¹²³I FP-CIT: Iodine-123 N-(3-fluoropropyl)-2beta-carbomethoxy-3beta-(4-iodophenyl) nortropane (FP- CIT)

¹¹C-VMAT: vesicular monoamine transporter

¹²³I β-CIT: Iodine-123 beta-CIT (2beta-carbomethoxy-3beta-(4-iodophenyl) tropane)

^{99m}Tc-TRODAT: Technetium-99m TRODAT

¹²³I-Altropane: Iodine-123 Altropane

(1.2) Literature Review of the P300 Wave in Schizophrenia Research – The P300 as a Neurobiological Marker in Schizophrenia

The P300 event related potential

Electrophysiology, a non-invasive imaging tool with outstanding temporal resolution, offers an ideal method to investigate cognitive processes and psychopathology in vivo. Event Related Potentials (ERPs) are changes in the electroencephalogram (EEG) that are typically observed after a certain stimulus or cognitive task is performed (Polich, 1998; Mathalon *et al.*, 2000b; Polich, 2007; Sumich *et al.*, 2013). The P300 event-related potential (ERP) in particular, is a positive deflection of the electroencephalogram peaking about 300 to 500 milliseconds after a participant receives an attended unusual or a task-relevant stimulus. The P300 is elicited by infrequent task-relevant stimuli embedded in a series of repeated presentations of a standard stimulus (Polich, 1998; Duncan *et al.*, 2009). The P300 wave is thought to reflect the cognitive process triggered by attending to selected sensory stimuli. It is effectively an EEG

correlate of sustained attention and working memory processes (Picton, 1992; Polich, 2007).

The P300 is one of the best characterised physiological biomarkers in neuropsychiatric research. The P300 amplitude is widely interpreted as a physiological correlate of cognitive resources allocated to processing the task-relevant stimulus; in other words, a measure of cortical activation during attentional efforts (Curran, 2004; Azizian & Polich, 2007). The P300 latency is classically interpreted as a central correlate of reaction time and is thought to reflect the speed of neural transmission (Picton, 1992; Polich, 2007). There are two major sub-components that have been well investigated. The P300a is produced without a task when an infrequent distractor in a series of frequent stimuli is presented. It is related to automatic attention processing and more frontally distributed. The P300b is elicited by infrequent and task-relevant target stimuli during an oddball paradigm. The P3b is most prominent in parietal regions and is thought to reflect the weight of attentional resources allocated to the detection of target stimuli (Azizian & Polich, 2007; Onitsuka *et al.*, 2013).

The P300 signal is generated from the summation of activity from multiple brain sources, particularly the various association areas of the cerebral cortex and the limbic system (Picton, 1992; Bledowski *et al.*, 2004; Polich, 2007; Mangalathu-Arumana *et al.*, 2012). Intracranial recordings have indicated that the P300 is a component with multiple generators including the prefrontal, anterior cingulate, superior temporal and parietal cortices as well as the hippocampus (Halgren *et al.*, 1998; Onitsuka *et al.*, 2013). However, despite a considerable body of literature, the neurochemical substrates of the P300 and the specific neurotransmitter systems involved in generating this ERP response remain poorly understood (Picton, 1992; Frodl-Bauch *et al.*, 1999a; Nieuwenhuis *et al.*, 2005;

Polich & Criado, 2006). Studies using multi-modal imaging methods on the same study participants are needed to help us to elucidate the nature of cognitive deficits and symptoms characterising psychosis and this is one of the objectives of this thesis.

P300 impairments in schizophrenia

Extensive research has shown that patients with schizophrenia have P300 amplitude reductions and latency prolongations compared to healthy controls (Frangou *et al.*, 1997; Coburn *et al.*, 1998; Ozgurdal *et al.*, 2008; Shin *et al.*, 2010; Jahshan *et al.*, 2013). As briefly mentioned in chapter 3, two independent meta-analyses have summarized the literature examining the P300 waveform in schizophrenia and found large effect sizes for this event related potential. The latest of these meta-analyses included over 45 original studies yielding a total sample size of more than 1400 patients and more than 1250 controls. From this work we know that the pooled standardised effect sizes (measured as Cohen's *d*) were 0.85 for the P300 amplitude reductions and 0.57 for the P300 latency delays in patients with schizophrenia when compared with healthy controls. These are respectively large and moderate-large effects and are comparable to some of the most robust findings in the neuroimaging and neuropsychological literature in schizophrenia (Jeon & Polich, 2003; Bramon *et al.*, 2004b).

There are however some limitations from the P300 schizophrenia literature. There was evidence of publication bias affecting the P300 amplitude and this means the true effect size might not be quite as strong as 0.8. There was no evidence of publication bias with regards to the moderate-large P300 latency deficits (Bramon *et al.*, 2004b).

Furthermore, this is a common caveat affecting many neuroimaging studies, the majority of EEG/ERP experiments available have been conducted with chronic patients treated with a range of antipsychotic, antimanic and other psychotropic medications. All these drugs are well known to cross the blood brain barrier and to influence the human EEG (Ford *et al.*, 1994a; Mathalon *et al.*, 2000a; Bramon *et al.*, 2004b; Molina *et al.*, 2004) and constitute plausible confounders to the proposed impairments typically attributed to the disease. Of course, there have also been a substantial number of EEG studies testing only patients who were medication free at the time of EEG recording, however these are rarely antipsychotic naïve participants (Bramon *et al.*, 2004b). Indeed, although the number of available studies is much smaller, antipsychotic free patients with schizophrenia have also been shown to have significantly attenuated P300 amplitude and delayed latency on oddball paradigms (Coburn *et al.*, 1998; Bramon *et al.*, 2004b). This evidence on medication-free patients offers reassurance against the important criticisms of medication confounding.

A wide range of antipsychotic drugs are licensed to treat schizophrenia and their potentially diverse effects on the P300 wave are not well understood. Although commonly prescribed antipsychotic drugs such as Olanzapine have been found to increase P300 amplitude in some studies (Mathalon *et al.*, 2000a; Bramon *et al.*, 2004b; Molina *et al.*, 2004), after treatment the P300 performance is generally not restored to normal levels (Ford *et al.*, 1994b; Hirayasu *et al.*, 1998; Jeon & Polich, 2003). On the contrary, a single study showed that the antipsychotic Sulpiride increased the P300 latency (Takeshita & Ogura, 1994). Finally, a recent meta-analysis of P300 changes among more than 500 Chinese patients with schizophrenia before and after antipsychotic use showed the

antipsychotic treatment is associated with a small but significant increase in the amplitude and decrease in the latency of P300 (Su *et al.*, 2012).

The P300 as a putative biomarker of psychosis risk

Compared to controls, a P300 amplitude reduction and latency delay was not only noted in patients with schizophrenia but also in their unaffected relatives such as siblings or offspring investigated as part of family studies (Ebmeier *et al.*, 1990; Shajahan *et al.*, 1997; Blackwood *et al.*, 1999; Bramon *et al.*, 2005; Ozgur dal *et al.*, 2008; Sumich *et al.*, 2008a; Jahshan *et al.*, 2013). These findings lend support to the P300 event-related potential being a potential biomarker of genetic predisposition towards developing schizophrenia (Bramon *et al.*, 2005; Turetsky *et al.*, 2015). Indeed, there is growing interest in using P300 and other similar EEG measures as alternative phenotypes for large multi-centre genetic association studies in psychosis (Ranlund *et al.*, 2014; Turetsky *et al.*, 2015; Light *et al.*, 2015).

Neurophysiological changes such as the reduction in P300 amplitude have also been described in populations at high risk of developing schizophrenia who present with “prodromal” symptoms, that is short-lived or sub-clinical psychotic experiences (van der Stelt *et al.*, 2005; Bramon *et al.*, 2008; Frommann *et al.*, 2008; van Tricht *et al.*, 2010). In cross-sectional studies, these P300 amplitude deficits ran a progressive course, from the prodromal to the chronic phases of schizophrenia (Ozgur dal *et al.*, 2008). However, a more recent longitudinal study shows that these impairments remain stable from the “prodrome” sub-clinical phase until after a full psychotic episode develops (van Tricht *et al.*, 2011). Sensory gating, mismatch negativity (MMN), and P300 have all been

demonstrated to be impaired in subjects clinically at risk of developing a psychotic disorders. Amongst these EEG measures, the duration of MMN showed the largest effect size for a difference between converters and non-converters of psychosis in a recent meta-analysis (Bodatsch *et al.*, 2015). In one recent study the P300 amplitude in combination with clinical parameters showed promising performance as a predictor of which individuals at risk for psychosis are more likely to develop the disease (Nieman *et al.*, 2014). Similarly, P300 amplitude and latency constitute useful alternative phenotypes (endophenotypes) for genetic association studies of psychosis (Turetsky *et al.*, 2015).

Given the literature reviewed from family studies as well as from other populations at increased risk of developing schizophrenia, P300 measures from auditory oddball paradigms are thought to be vulnerability indicators for schizophrenia. However, the influences of antipsychotics on ERP measures are poorly understood and medication confounding in case-control cross sectional studies remains a plausible and serious problem. Longitudinal studies are one way of clarifying such effects. We therefore set out to do a study with antipsychotic naïve patients with schizophrenia and after a pre-treatment baseline assessment we are in our fifth year of follow up.

The effects of age on the P300 waveform

P300 amplitude reduction with normal aging has been shown in some studies (Picton *et al.*, 1984; Ford & Pfefferbaum, 1991; Polich, 1991; Iragui *et al.*, 1993). There is clear evidence that P300 latency decreases during the first years of life (Polich *et al.*, 1990; Rozhkov *et al.*, 2009), whereas in older adults the parietal P300 latency increases, and the parietal P300 amplitude declines with advancing

age (Rossini *et al.*, 2007; Ashford *et al.*, 2011). The P300 follows a specific trajectory across the lifespan reflecting brain maturation in childhood and adolescence and degenerative effects in older age (van Dinteren *et al.*, 2014; Tsolaki *et al.*, 2015).

As noted, reduction of P300 amplitude has been found to be significant in drug-naïve and first episode schizophrenia patients (de Wilde *et al.*, 2008), but importantly, P300 amplitude negatively correlated with age in schizophrenia patients (Wang *et al.*, 2003). Although the prolongation of P300 latency with age occurred in both groups, the regression slope for P300 latency with age was significantly steeper in patients with schizophrenia than in normal controls (Wang *et al.*, 2003).

As is the case for most cognitive performance measures (Polich, 2007), the P300 waveform is influenced by age. The existing literature shows that the effects of ageing are in the same direction as the illness-related effects (P300 amplitude reductions and latency delays associated with both ageing and schizophrenia). Therefore in my thesis I will examine in detail the potential contribution of ageing to any difference in clinical groups.

Sex influences on the P300 response

Significant sex-related differences have been found in the response time on the behavioural side (button press speed in response to targets) of the P300 ERP among healthy individuals, with males showing faster responses than females (Tsolaki *et al.*, 2015). Similarly, the level and location of the maximum current intensity of the P300 were also noted to differ between sexes, revealing males had larger current intensity than females (Tsolaki *et al.*, 2015). Furthermore, the P300

amplitude has also been noticed to be greater in healthy females than in healthy males (Schiff *et al.*, 2008).

In younger control populations, girls showed greater bilateral frontal P300 amplitudes, approaching the higher values observed in boys during childhood. Right frontal P300 amplitude has been found to be negatively associated with reaction time in girls after controlling for age (Sumich *et al.*, 2012), that is in line with previous studies which suggested females rely more on frontal activations than males during cognitive tasks (Christakou *et al.*, 2009). These findings have demonstrated sex differences in ERP maturation in line with behavioural and neuroimaging studies. Reduced auditory P300 amplitude is one of the most robust electrophysiological findings in schizophrenia (Turetsky *et al.*, 2009). Sex differences for P300 (i.e. lower amplitude in male patients) appear to be preserved in schizophrenia (Sumich *et al.*, 2013). Among patients with schizophrenia, men showed lower novelty P300 amplitude than women, and particularly in women and over the right hemisphere, novelty P300 amplitude was inversely associated with excitement (Sumich *et al.*, 2013). Furthermore, depression in patients with schizophrenia was significantly positively associated with early perceptual processing in response to novel stimuli in men (parietal N100 amplitude), and with a later processing stage (parietal P3b) in women (Sumich *et al.*, 2014).

Therefore, the existing literature shows that the effects of sex on P300 amplitude in patients with schizophrenia are comparable to the sex differences observed in ERP maturation of healthy individuals. Thus amongst people with schizophrenia, the males have lower P300 amplitudes than the females. Therefore, in my thesis I intend to examine in detail the potential contribution of sex to any difference I observe between the clinical groups.

The relationship between P300 and psychopathology

Several measures of schizotypy have been associated with electrophysiological measures of brain function, and further shown to be modified by sex. Paranormal ideation (PI) was inversely associated with P300 amplitude at right-anterior electrodes in women, and right-anterior P300 amplitude was negatively associated with unusual experiences (UEs) only in women. These results provide support from electrophysiological measures which were modified by sex for the dimension of schizotypy, and lend indirect support to dimensional conceptions of psychotic and other mental disorders (Sumich *et al.*, 2008b).

In terms of the relationship with symptoms, P300 subcomponents are affected in a different manner by positive versus negative symptoms in patients with schizophrenia (Frodl-Bauch *et al.*, 1999b). The auditory P300 amplitude recorded in the left hemisphere has been proposed as a potential state marker to reflect the severity of positive symptoms and that these positive symptoms may be caused by a possible left-hemisphere deficit in schizophrenia (Higashima *et al.*, 2003). Auditory P300 amplitudes could track clinical fluctuations and are considered a state marker of schizophrenia in some studies. For example P300 amplitude tracked Brief Psychiatric Rating Scale (BPRS) total scores and positive symptom scores over time, with decreasing amplitude during symptom exacerbations and increasing amplitude associated with improvements in psychopathology (Mathalon *et al.*, 2000a). Given the existing literature mostly on treated patients, I will examine the relationship between symptom severity (measured by PANSS) and P300 amplitude and latency in my sample of drug naïve people with schizophrenia.

The P300 response and dopamine function in schizophrenia

Only a handful of studies have attempted to investigate the relationship between dopamine function and P300 performance and its resulting implications for schizophrenia (Kenemans & Kahkonen, 2011). The interactions of dopaminergic with other neurotransmitter pathways constitute a considerable challenge for this endeavour (Peters *et al.*, 2004; Ford *et al.*, 2007).

According to Pogarell *et al.* (2011), D₂/D₃ receptor availability positively correlates with P300 amplitude and negatively correlates with P300 latency. Therefore, central dopaminergic activity might play an important role in the generation and modulation of the P300 response (Pogarell *et al.*, 2011). Dopamine is thought to be critical for the cognitive response to novelty detection. In their experiments with healthy volunteers, Rangel-Gomez *et al.* (2013) manipulated dopamine activity using Apomorphine (a D₁/D₂ agonist) and measured the change in several neurological indices of novelty processing. Apomorphine speeded and potentiated the novelty-elicited N2, an ERP component thought to index early aspects of novelty detection, and caused novel words to be better recalled. Apomorphine also decreased the amplitude of the novelty P300a waveform. Thus an increase in D₁/D₂ receptor activation appears to potentiate neural sensitivity to novel stimuli, causing this content to be better encoded (Rangel-Gomez *et al.*, 2013).

A range of neurotransmitter systems are thought to influence the P300 waveform. Dopamine neurotransmission is thought to be of importance in both perceptual processing and motor preparation (Kenemans & Kahkonen, 2011). Nieuwenhuis *et al.* indicated a stimulating contribution of the ascending

noradrenergic locus–coeruleus system in generating the P300 response, based on their review of both animal and human research (Nieuwenhuis *et al.*, 2005). Furthermore, the P300 as the prototypical attentional response to relevant or salient stimuli has been found to be impaired not only in schizophrenia, but in a range of mental health disorders where catecholaminergic deficits play a role such as mood disorders and addictions (Jonkman *et al.*, 2000).

Further investigation of the interaction between P300 ERP and DAT function could help us to understand the dopaminergic pathophysiology of schizophrenia and this is one of the aims of my thesis.

(1.3) Aims and Hypotheses

This thesis aims to:

- (i) Collect a new sample of drug naïve patients with schizophrenia and examine their DAT availability and P300 ERP performance (and other psychopathology ratings).
- (ii) Investigate the relationship between DAT availability and P300 performance in drug-naïve schizophrenia.
- (iii) Examine the published evidence from case control studies and conduct meta-analyses of DAT availability and P300 performance in drug naïve schizophrenia.

The following hypotheses will be tested:

- DAT availability will be reduced in drug naïve patients with schizophrenia compared to healthy controls.
- The P300 wave will have reduced amplitude and delayed latency in drug

naïve patients with schizophrenia compared to healthy controls.

Chapter 2 of this thesis focuses on the role of DA pre-synaptic regulation. I compared DAT availability using [^{99m}Tc]-TRODAT-1 SPECT between drug naïve patients with recent onset schizophrenia and healthy controls in the largest study to date. I then conducted a meta-analysis of all studies that used SPECT imaging, including my new data, to compare DAT availability in antipsychotic naïve patients with schizophrenia versus controls to determine if there is evidence of altered DAT availability.

Chapter 3 of this thesis explored ERP differences between drug naïve patients with schizophrenia and healthy controls to examine if P300 performance is related to dopamine transporter (DAT) availability, without the confounding effects of medication. This was made possible since a subset of the participants in this study volunteered to undergo both single photon emission computed tomography (SPECT) with [^{99m}Tc]-TRODAT-1 as well as EEG testing. I then conducted an up-dated meta-analysis of studies that used P300 ERP, including my new data, to compare P300 in antipsychotic naïve patients with schizophrenia against controls to determine if there is evidence of altered P300 in drug naïve patients with schizophrenia. I predicted that P300 amplitude would positively correlate with DAT availability while latency would have a negative correlation with DAT availability in patients with schizophrenia (Bramon *et al.*, 2004b; Chen *et al.*, 2013).

Chapter 2

Striatal dopamine transporter availability in drug naïve patients with schizophrenia: a case-control SPECT study with [^{99m}Tc]-TRODAT-1 and a meta-analysis

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Striatal Dopamine Transporter Availability in Drug-Naïve Patients With Schizophrenia: A Case-Control SPECT Study With [^{99m}Tc]-TRODAT-1 and a Meta-Analysis

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Central dopaminergic hyperactivity has been one of the main hypotheses of the pathophysiology of schizophrenia since the 1970s. Excess dopamine (DA) neurotransmission in the striatum is hypothesized to alter the processing of information and result in psychotic symptoms in schizophrenia. Single photon emission computerized tomography (SPECT) provides in vivo indices of DA neurotransmission. Our study aimed to compare dopamine transporter (DAT) availability between drug-naïve patients with schizophrenia and controls using SPECT. DAT availability through [^{99m}Tc]-TRODAT-1 SPECT was compared between 47 drug-naïve patients with recent-onset schizophrenia and 112 healthy controls. We also conducted a random-effects meta-analysis of the available literature synthesizing the results of 6 comparable published articles as well as our current data. The mean specific striatal binding showed a statistical trend for a reduction among the patients compared with controls (estimated difference = 0.071; 95% CI -0.01, 0.15; *P* = .08). There was an effect of gender, whereby females had a higher ratio of specific striatal binding than males. Age was negatively correlated with the ratio of specific striatal binding, both in patients and controls. The meta-analysis provided a pooled standardized effect size (Cohen's *d*) of -0.07 (95% CI -0.31, 0.18; *P* = .60) for the patient vs control comparison in TRODAT binding, with no evidence of heterogeneity between studies or publication bias. Our findings suggest that striatal DAT levels are not altered in the early stages of schizophrenia before medication is introduced. We identified gender differences and aging effects that could have significance for future studies.

Key words: drug-naïve schizophrenia/dopamine/dopamine transporter/TRODAT/single photon emission tomography/meta-analysis

Introduction

Central dopaminergic hyperactivity has been one of the main hypotheses of the pathophysiology of schizophrenia since the 1970s.^{1,2} Although it has undergone a number of revisions, in its current form, excess dopamine (DA) neurotransmission in the striatum is hypothesized to alter the processing of information and result in psychotic symptoms.^{2,3} Neurochemical imaging techniques such as single photon emission computerized tomography (SPECT) and positron emission tomography (PET) provide in vivo indices of the stages of DA neurotransmission, including its presynaptic synthesis, DA release into the synapse, and the levels of DA receptors and dopamine transporters (DATs).²

Elevated DA synthesis capacity has been consistently reported in 6-fluoro-(18F)-L-3,4-dihydroxyphenylalanine ([18F]-DOPA) and L-[β-11C]-3,4-dihydroxyphenylalanine ([11C]-DOPA) PET studies in schizophrenia, including in the first episode,^{3,4} and has been shown to predate the onset of schizophrenia in individuals with prodromal psychotic symptoms.⁵ Synaptic DA can be studied using challenge approaches that stimulate DA release or deplete synaptic DA levels. These approaches are based on the competition between DA and radioligands such as raclopride and [¹²³I]iodobenzamide for binding to DA receptors,⁶ although

recent evidence indicates the process is more complex than suggested by a simple competition model.⁷ Studies using challenge approaches have found evidence of increased radiotracer displacement in patients with schizophrenia compared with controls, indicating greater DA release,⁸ and increased synaptic DA levels.^{9,10} DA neurotransmission in the striatum is largely terminated by the reuptake of DA into the presynaptic DA nerve terminals by DATs. Thus, alterations in DAT availability could also alter DA neurotransmission and contribute to the pathoetiology of schizophrenia. DAT availability has been investigated in schizophrenia with evidence of a decrease in some studies^{11,12} but no difference or an increase in others.^{13–16} A recent review found that there was significant between-study heterogeneity, which may be due to the small sample sizes and the inclusion of patients at different stages and on different treatments. Howes OD et al (in preparation) There is therefore a need for a large study of drug-naive first-episode patients to determine whether there are DAT abnormalities associated with the onset of the illness.

Our study focused on the role of DA presynaptic regulation. We compared DAT availability using [^{99m}Tc]-TRODAT-1 SPECT between 47 drug-naive patients with recent-onset schizophrenia and 112 healthy controls in the largest study to date. We then conducted an updated meta-analysis of studies that used SPECT imaging, including our new data, to compare DAT availability in antipsychotic-naive patients with schizophrenia with that in controls to determine if there is evidence of altered DAT availability in drug-naive patients with schizophrenia.

Methods

Sample

All study participants were living in Tainan City, the fifth largest in Taiwan with a population of 1,873,579. A total of 47 drug-naive first-episode patients with schizophrenia were recruited at the psychiatric outpatient clinic of the National Cheng Kung University Hospital. One hundred and twelve healthy community residents of Tainan City were also recruited through research advertisements. They were recruited to enable the study of factors underlying normal variation in DAT activity and as controls for a number of planned psychiatric and neurological research studies. These controls were interviewed by senior psychiatrists who had been practicing for more than 10 years, using the Chinese version of the Mini International Neuropsychiatric Interview,¹⁷ to ensure that the subjects were free of any Axis I or Axis II psychiatric disorders. These subjects were physically healthy and without any history of alcohol or other substance abuse or dependence. Any subject who had been taking psychotropic medications, including antipsychotics or antidepressants, were excluded. Brain magnetic resonance images (MRIs) and routine blood tests done in the controls were normal. All the participants recruited in our previous study,¹⁴ 11

patients and 12 controls, were also included in the present local study. The mean duration of illness was 15.8 months (SD = 33.5, median = 5.4, interquartile range = 16.5).

Before any procedure was performed, written informed consent was obtained from each of the participants after a complete explanation of the study. The Ethical Committee for Human Research at the National Cheng Kung University Hospital approved the study protocol. Inclusion criteria for all participants were as follows: (1) patients should fulfill *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* (DSM-IV) criteria for schizophrenia; (2) age between 18 and 60; (3) no physical illness and with stable vital signs and no evidence of substance abuse/dependence as assessed during the clinical interview with the research psychiatrist at the time of enrollment; (4) never received any antipsychotic or antidepressant treatment and were free of any psychotropic medication at the time of testing. These patients had never had such psychotropic medications prescribed, and our clinic was their first contact with psychiatric services. Exclusion criteria for all participants were as follows: (1) other comorbid psychiatric illnesses, substance abuse/dependence, or neurological illnesses; (2) mental retardation; (3) all female participants of childbearing age had to take an acceptable form of contraceptive throughout the duration of the study in order to be included and all female participants underwent an instant urine pregnancy test prior to starting the experiment; (4) all patients who were deemed at risk of acute suicide/self-harm were excluded from the study for their safety.

Assessment Battery

Before receiving any treatment, the patients underwent all the baseline assessments within 7 days of entering the study. The healthy controls received the same assessments.

[^{99m}Tc]-TRODAT-1 SPECT and MRI. Each subject received a bolus intravenous injection of 740 MBq (20 mCi) of [^{99m}Tc]-TRODAT-1 (Institute of Nuclear Energy Research, Lungtan, Taiwan) in a quiet environment approximately 10 minutes after the intravenous line was set up. The brain SPECT images were acquired 4 hours after the injection. To avoid tilt and misalignment of the participant's head in the image scanner, we carefully positioned participants and monitored them during scanning and used a head holder to further reduce movement artifacts. Before image acquisition, the participant was informed of the necessity to avoid head movement. Sinograms were reviewed blind to diagnosis to determine whether postacquisition correction for head movements was needed. Movement correction was conducted by using the motion correction software ICON (Siemens, version 8.5 KB21).

We used a triple-headed rotating gamma camera (Multispect 3; Siemens, Hoffman Estates, IL, USA) with ultra high-resolution fan-beam collimators, which yields an

image resolution of approximately 8.5 mm for the full-width half maximum (FWHM). The SPECT images were acquired over a circular 360° rotation, with 120 steps, at a rate of 50 seconds per step, in a 128 × 128 × 16 matrix. The images were then reconstructed using Butterworth and Ramp filters¹⁸ (cutoff frequency = 0.3 Nyquist, power factor = 8), with attenuation according to Chang's method.¹⁹ The reconstructed transverse images were realigned parallel to the canthomeatal line. The slice thickness of each transverse image was 2.89 mm. For semiquantitative analyses, 6 consecutive transverse slices on which the highest striatum uptake was best visualized were combined to obtain a 17.34-mm-thick slice. Then regions of interest (ROIs) were placed over the striatum and the occipital cortex. The ROIs were drawn directly on the SPECT images by an experienced nuclear medicine physician who was blind to the participants' clinical data. The participants' MRIs (SIGNA CV-I, 1.5 T; GE, USA), obtained within 2 weeks after SPECT examination, were used as a visual reference to determine the ROIs. The sizes of all ROIs were at least twice that of the FWHM. The specific striatal [^{99m}Tc]-TRODAT-1 binding (which represents striatal DAT availability) was calculated as the mean count in the striatal ROI divided by the mean count in the occipital region (St/Oc).²⁰

Psychopathology Ratings. On the day of recruitment, standardized psychopathology ratings using Clinical Global Impression Severity of Illness (CGI-S),²¹ Global Assessment of Functioning (GAF; range 0–100 from poorest to optimal functioning),²² and the Structured Clinical Interview for Positive And Negative Symptoms Scale (SCI-PANSS; range 30–210 from least to most symptomatic)²³ were collected for all patients.

Statistical Analyses

Local Study. The main aim of our study was to assess the differences between patients and controls in the specific striatal [^{99m}Tc]-TRODAT-1 binding, considering both the left and right striatum measures. Mixed modeling was used to compare [^{99m}Tc]-TRODAT-1 binding between the 2 clinical groups, and to allow for possible group differences between left and right sites, we tested if a group by laterality interaction was evident. On the basis of previous literature, we considered that age, sex, and tobacco smoking are potential confounders and therefore included these as covariates in the analysis.^{24–26} The models included subject-varying intercepts to acknowledge correlation between the 2 repeated measures per participant.

The second aim of this study was to evaluate the effects of aging, sex, and tobacco smoking on the specific striatal [^{99m}Tc]-TRODAT-1 binding, and we present the regression coefficients obtained from the model described above. Our third and final objective was to use the data to assess if any group effect varies with age or gender. Thus, we

expanded the above model to test an interaction of group by age or group by gender, respectively.

The association between the specific striatal [^{99m}Tc]-TRODAT-1 binding and psychopathology ratings in patients was analyzed by using Spearman's rho correlations.

Demographic differences between patients and controls were examined with Chi-square tests for categorical variables or with Student *t* tests for continuous variables. For the latter, Levene's test was used to assess the assumption of equality of variances. Diagnostic plots as well as one-sample Kolmogorov-Smirnov tests were used to test for normality. Statistical significance was established at *P* < .05. SPSS version 16 (SPSS Inc., Chicago, IL, USA) was used for all analyses.

Meta-Analysis. We conducted a random-effects meta-analysis combining the previous published literature as well as the local study presented here. We searched the Institute for Scientific Information Web of Knowledge, Scopus, and PubMed (U.S. National Library of Medicine, NLM) using the following 2 sets of key words: (1) "drug-naïve, schizophrenia, dopamine transporter" and (2) "drug naïve, schizophrenia, TRODAT, dopamine transporter." The search covered between 1960 and the end of 2010 and yielded a total of 40 articles. Of these, we excluded 3 reviews, 4 conference abstracts, 2 animal studies, 8 basic/genetic studies, 8 articles of other psychiatric illnesses, 2 articles on medicated patients with schizophrenia, and 7 articles on ligands different from TRODAT. Thus, the meta-analysis finally included 6 articles published between 2003 and 2010 that focused on drug-naïve schizophrenia patients using [^{99m}Tc]-TRODAT-1.

Our local data were included as the seventh study. The standardized mean difference between controls and patients was computed for each primary study as Cohen's *d*.²⁷ The meta-analysis, tests for effect moderation, and publication bias were conducted using, respectively, the metan, metareg, and metabias routines available in STATA 10 (Stata Corporation, College Station, TX, USA).

Results

Local Study

The sample included 47 drug-naïve patients with a DSM-IV diagnosis of schizophrenia as well as 112 healthy controls.

The demographic characteristics of the patient and control groups are summarized in table 1. Patients and controls had a similar gender and tobacco smoking status distribution. However, compared with the controls, the patients were significantly younger (*t* = −4.19, *df* = 117.7; *P* < .001), less likely to be married (χ^2 = 11.47; *P* = .001), and had fewer years of education (*t* = −2.62, *df* = 154; *P* = .01). The ratio of specific striatal binding in both patients and controls was normally

Table 1. Demographic Characteristics of the Participants

	Schizophrenia (<i>n</i> = 47) Mean (SD)	Normal Control (<i>n</i> = 112) Mean (SD)	Statistical Test		
			<i>t/χ²</i>	<i>df</i>	<i>P</i>
Age (years), range	27.2 (8.7), 17.0–52.7	34.3 (12.0), 18.9–58.8	−4.19	117.7	<0.001
Sex (male/female)	26/21	59/53	0.09	1	0.761
Smoking status (yes/no)	5/42	19/93	1.03	1	0.300
Marital status (M/S)	5/42	42/70	11.47	1	0.001
Years of education, range	12.6 (3.2), 5–21	14.2 (3.5), 0–22	−2.62	154	0.010

Note: M includes married and living with the partner; S includes single, divorced, and married but separated.

distributed ($P > .2$ and diagnostic plots). The group by laterality interaction was not significant and was therefore dropped from our model ($F = 2.02$, $df = 1, 157$; $P = .16$). After controlling for age, sex, and tobacco smoking, the mean specific striatal binding showed a trend for a difference between patients and controls, where patients had a reduction in their TRODAT binding (estimated difference between controls and patients = 0.071; 95% CI −0.01, 0.15; $F = 3.19$, $df = 1, 154$; $P = .08$). These results are summarized in table 2.

In the same model, there was a significant effect of gender, whereby females had a higher ratio of the specific striatal binding than males (estimated difference between males and females = −0.08; 95% CI −0.15, −0.002; $F = 4.11$, $df = 1, 154$; $P = .04$). Tobacco smoking did not have a significant influence on TRODAT binding (estimated difference between smokers and nonsmokers = −0.06; 95% CI −0.17, 0.04; $F = 1.39$, $df = 1, 154$; $P = .24$). Finally, there was a highly significant effect of age, whereby TRODAT binding declined with advancing age (estimated change per decade of age = −0.1; 95% CI −1.2, −0.6; $F = 31.87$, $df = 1, 154$; $P < .001$). Of note, there was no significant interaction between age and group ($F = 0.37$, $df = 1, 153$; $P = .55$) or between group and sex ($F = 0.20$, $df = 1, 153$; $P = .66$), indicating that the age decline in TRODAT was similar in patients and controls as well as in both genders. These findings are summarized in table 2 and figure 1.

Of the 47 patients included, 43 completed the GAF, 42 the PANSS, and 39 the CGI scales. No significant correlations were found between the specific striatal binding and psychopathological rating scores (table 3).

Systematic Review. To date, 6 SPECT studies with [^{99m}Tc]-TRODAT-1 have been published (table 4).^{13–15,28–30} Schmitt et al¹⁵ found no significant difference in [^{99m}Tc]-TRODAT-1 binding to the striatal DAT between 20 drug-naive patients and 12 normal controls, even though the controls seemed to have a larger mean value of TRODAT binding ratio than did patients in their another study.²⁸ Similarly, Hsiao et al¹³ reported no significant average differences in TRODAT uptake ratio between 12 drug-naive patients with schizophrenia and 12 controls; however, the patients showed a lack of right-left asymmetry in striatal uptake of TRODAT. Finally, our previous study with 11 patients and 12 controls¹⁴ and the study of Chou et al³⁰ with 7 patients and 11 controls also failed to identify any deficits among drug-naive patients. The largest study in our review,²⁹ with 28 patients and 12 normal controls, was again with no significant differences of DAT availability between patients and controls. However, the authors highlighted that in a subsample of 18 patients with a positive syndrome as defined by Kay,²³ the severity of hallucinations was inversely correlated with DAT availability. This finding differs from the rest of the studies we

Table 2. The DAT Availability (St/Oc) by Group and by Sex

	<i>n</i>	Total		Right		Left	
		Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range
Group							
Schizophrenia	47	2.20 (0.25)	1.75–2.81	2.20 (0.26)	1.80–2.90	2.19 (0.24)	1.70–2.73
Normal control	112	2.20 (0.24)	1.57–2.71	2.20 (0.24)	1.57–2.71	2.20 (0.24)	1.58–2.71
Sex							
Male	85	2.17 (0.23)	1.73–2.66	2.17 (0.24)	1.73–2.71	2.17 (0.23)	1.67–2.67
Female	74	2.23 (0.25)	1.57–2.81	2.23 (0.25)	1.57–2.90	2.24 (0.25)	1.58–2.73

Note: DAT, dopamine transporter.

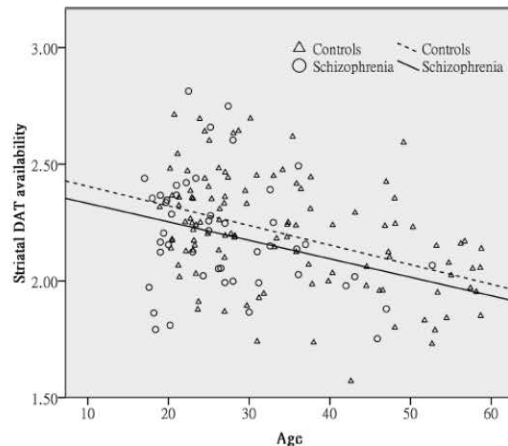


Fig. 1. The relation between age and striatal DAT availability in patients with schizophrenia and controls. As described in the main analyses, having adjusted by the main effects of group, sex, and tobacco smoking, there was a significant decline of TRODAT binding with advancing age but no significant interaction between the age and group. This graph shows regression lines describing the relationship between TRODAT binding and age within patients and control samples separately. The almost parallel lines illustrate the similar rates of decline in the 2 groups. DAT, dopamine transporter.

reviewed that showed no evidence of correlation between symptoms and DAT availability.^{13–15,28}

Meta-Analysis

We conducted a random-effects meta-analysis of the available literature including 6 previously published articles as well as our current data. To avoid including them twice, our current data here excluded 11 patients and 12 controls recruited in our previous study¹⁴ for the meta-analysis. The average ROI value of TRODAT

binding over left and right hemispheres was used for studies where this information was available. The combined sample included a total of 124 drug-naïve patients with schizophrenia and 169 healthy controls. The pooled standardized effect size computed as a Cohen's d ²⁷ was -0.07 (95% CI $-0.31, 0.18$; $P = .60$) indicating that patients and controls do not differ significantly. There was no significant between-study heterogeneity in effect sizes (coefficient = -0.07 ; $P = .61$). Figure 2 provides a forest plot for the meta-analysis. Furthermore, there was no evidence of publication bias (Egger's test coefficient = 0.90 ; $P = .28$).

Discussion

Our findings show that the specific striatal binding ratio of drug-naïve patients with schizophrenia was not significantly different from that of normal controls, indicating that both groups had similar DAT availability. Our study is the largest to date, including over twice the sample of drug-naïve/drug-free patients of the next largest study of DAT in schizophrenia.³¹ Another strength of our study is that all the subjects were drug naïve and at a uniform and early stage of their illness. Our findings thus extend previous work in a larger and clinically homogenous sample. They are consistent with all the previous SPECT studies of schizophrenia using [^{99m}Tc]-TRODAT-1 that were included in our meta-analysis.^{13–15,28–30}

The meta-analysis including our data found there was no evidence for a difference in striatal [^{99m}Tc]-TRODAT-1 binding in drug-naïve patients with schizophrenia compared with controls. Considering the range of effect sizes included in the 95% CI, the standardized effect size is unlikely to exceed 0.31, which is conventionally considered to be a small effect.²⁷ With this effect size, as many as 188 drug-naïve patients and an equal number of controls would need to be tested to have 85% power to detect a difference (two-group T test with a 5% two-sided significance level). The overall conclusion of our work is that

Table 3. Spearman's Rho Correlations of Striatal DAT Availability and Psychopathology in the Patient Group

	PANSS				CGI-S	GAF
	Positive	Negative	General Psychopathology	Sum		
<i>n</i>	42	43	43	42	39	43
Mean (SD)	22.0 (6.2)	20.1 (7.1)	37.2 (11.4)	79.3 (21.6)	4.7 (1.0)	41.1 (15.0)
Range	7–34	8–42	16–62	32–138	2–6	5–75
Striatal DAT availability						
ρ	0.25	0.05	0.17	0.15	0.17	0.18
P	0.11	0.74	0.27	0.35	0.32	0.25

Note: DAT, dopamine transporter; PANSS, Positive and Negative Syndrome Scale; CGI-S, Clinical Global Impression Severity of Illness; GAF, Global Assessment of Functioning.

Table 4. Summary of All [^{99m}Tc]-TRODAT-1 Relevant Published Articles

Patients			Controls				Psychopathological rating			DAT and DA study receptor only		Summary		
			Age		Matched								Age	
Authors	Year	Journal	n	Male/female	Mean	SD	n	Matched	Male/female	Mean	SD			
Chou et al	2010	<i>Clinical Psychopharmacology and Neuroscience</i>	7	—	22.4	5.3	11	Age	—	25.2	4.5	Nil	Yes	No difference in striatal DAT availability between patients and controls
Schmitt et al	2008	<i>Schizophrenia Research</i>	20	18/2	29.3	6.5	12	Age and sex	9/3	30.5	8	CGI, GAF, and PANSS	Yes	No difference in striatal DAT availability between patients and controls
Schmitt et al	2006	<i>European Archives of Psychiatry and Clinical Neuroscience</i>	28	23/5	30.7	8.9	12	Age	10/2	31.7	8.4	CGI, GAF, and PANSS	Yes	No difference in striatal DAT availability between patients and controls
Schmitt et al	2005	<i>Journal of Psychopharmacology</i>	10	6/4	34.9	12.1	10	Age and sex	—	37.8	10.8	BPRS, PANSS, and SANS	Yes	No difference in striatal DAT availability between patients and controls
Yang et al	2004	<i>American Journal of Psychiatry</i>	11	6/5	26.3	10.2	12	—	9/3	33.3	12.9	PANSS	Yes	No difference in striatal DAT availability between patients and controls
Hsiao et al	2003	<i>Schizophrenia Research</i>	12	2/10	25.9	7.7	12	Age and sex	—	29.8	8.6	SCAN and PANSS	Yes	No difference in striatal DAT availability between patients and controls

Note: DAT, dopamine transporter; DA, dopamine; CGI, Clinical Global Impression; GAF, Global Assessment of Functioning; PANSS, Positive and Negative Syndrome Scale; BPRS, Brief Psychiatric Rating Scale; SANS, Schedule for Assessment of Negative Symptoms; SCAN, Standardized Clinical Assessment for Neuropsychiatry.

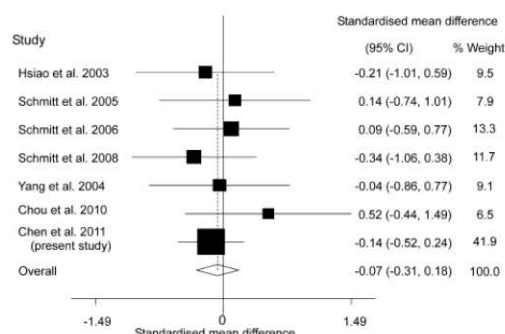


Fig. 2. Meta-analysis of TRODAT literature and local study focusing on drug-naïve patients.

drug-naïve patients in the early stages of schizophrenia have normal [^{99m}Tc]-TRODAT-1 binding and that any impairments, if present, would be of a small magnitude. Given the normal variation in binding, any potential deficits in drug-naïve patients are unlikely to be clinically significant. Although [^{99m}Tc]-TRODAT-1 binds to serotonin transporters, blocking studies show that the specific binding in the striatum is almost entirely due to DATs³²; hence, our findings reflect that DAT availability is unaltered in drug-naïve patients with schizophrenia.

However, it remains conceivable that there is a transient alteration in DAT at or before the onset of the disorder that may have been missed by all of the above studies including our own because our patients probably suffered from psychotic symptoms for months or even years prior to their SPECT scan.

Methodological Considerations

We need to consider the possible influence of tobacco smoking because a decrease in DAT availability in the striatum of smokers has been described in previous studies.²⁶ A study of nonsmokers only would not be representative of most clinical populations, and we included participants regardless of their smoking habit. Our patient and control groups were not significantly different in smoking status; hence, smoking is unlikely to have confounded our results. Nevertheless, all our analyses were adjusted for tobacco smoking in this sample.

One potential limitation of our study is that the ROIs were manually drawn directly on the SPECT image. While there is the potential for observer bias using this approach, we avoided this by ensuring that the ROIs were delineated by an experienced nuclear medicine physician independent of the clinical assessment and blind to the subject's clinical group. We delineated the ROI on the SPECT image rather than on the MRI because this does not require the ROI to be transformed from MRI to SPECT space. However, studies have found similar

results using both approaches^{33,34} indicating that the choice of approach is unlikely to have influenced our findings.

Another potential limitation is that patients recruited in our study for SPECT imaging had to be relatively cooperative in order to complete the procedure. This is an issue for most imaging studies and may potentially affect the generalizability of our findings to uncooperative patients.

Interpretation of Our Findings

The presynaptic DAT plays a key role in regulating the DA content in the synaptic cleft by transporting it back into DA terminals, effectively modulating the concentration of DA available for postsynaptic receptor binding.³⁵ There are 2 main interpretations of our findings: on the one hand, if DA was able to displace [^{99m}Tc]-TRODAT-1 from DAT, our failure to find a difference in specific binding could be due to elevated synaptic DA levels in schizophrenia masking an increase in DAT levels.¹⁰ However, while the definitive studies have not yet been conducted, the available evidence indicates that [^{99m}Tc]-TRODAT-1 binding is not sensitive to variations in DA levels seen *in vivo*.³⁶ A more likely interpretation is thus that DAT levels are unaltered in schizophrenia. This is important because it indicates that there is no compensatory increase in DAT levels in response to the increased release and synaptic levels of DA previously reported in schizophrenia.^{8,10} This interpretation is consistent with evidence that fluctuations in brain DA levels do not alter the abundance of the DAT.³⁷ Our findings might thus suggest pharmacological augmentation of DAT as a potential novel treatment strategy for schizophrenia.

We identified a gender effect in the combined sample, whereby females had a higher specific striatal radiotracer binding ratio than males, which is consistent with a previous study.²⁴ The effect size for gender in our study is 0.25 (Cohen's *d*) and although this is a small effect, it might be of relevance. It would seem to be in line with the later age of onset and lower incidence of schizophrenia in females.³⁸ The lower DAT availability in males could contribute to their earlier age of onset because, putatively, they would have less capacity to buffer excess striatal DA release.

Our finding that there is a negative relationship between age and DAT availability is consistent with a previous study showing that the specific uptake of [^{99m}Tc]-TRODAT-1 radiotracer decreases with advancing age in healthy individuals.²⁵ Because the density of striatal DA D₂/D₃ receptors also declines with age in healthy individuals,³⁹ our data show the consistency of this age effect on both pre- and postsynaptic DAergic function.

The PANSS scores for positive and negative symptoms of schizophrenia and general psychopathology did not correlate with the [^{99m}Tc]-TRODAT-1 binding ratio.

This is again consistent with previous research^{13,14,28} and with the absence of alterations in [^{99m}Tc]-TRODAT-1 availability in our patients as a group.

Conclusions

Our findings, in the largest first-episode drug-naive and healthy sample studied to date, suggest that striatal DAT availability is not altered in the early stages of schizophrenia before medication is introduced. Instead, we identified gender differences and confirmed aging effects that could have clinical significance and may be taken into account in future patient studies.

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Chapter 3

P300 waveform and dopamine transporter availability: a controlled EEG and SPECT study in medication naïve patients with schizophrenia and a meta-analysis

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ORIGINAL ARTICLE

P300 waveform and dopamine transporter availability: a controlled EEG and SPECT study in medication-naïve patients with schizophrenia and a meta-analysis

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Background. Reduced P300 event-related potential (ERP) amplitude and latency prolongation have been reported in patients with schizophrenia compared to healthy controls. However, the influence of antipsychotics (and dopamine) on ERP measures are poorly understood and medication confounding remains a possibility.

Method. We explored ERP differences between 36 drug-naïve patients with schizophrenia and 138 healthy controls and examined whether P300 performance was related to dopamine transporter (DAT) availability, both without the confounding effects of medication. We also conducted a random effects meta-analysis of the available literature, synthesizing the results of three comparable published articles and our local study.

Results. No overall significant difference was found in mean P300 ERP between patients and controls in latency or in amplitude. There was a significant gender effect, with females showing greater P300 amplitude than males. A difference between patients and controls in P300 latency was evident with ageing, with latency increasing faster in patients. No effect of DAT availability on P300 latency or amplitude was detected. The meta-analysis computed the latency pooled standardized effect size (PSES; Cohen's *d*) of -0.13 and the amplitude PSES (Cohen's *d*) of 0.48 , with patients showing a significant reduction in amplitude.

Conclusions. Our findings suggest the P300 ERP is not altered in the early stages of schizophrenia before medication is introduced, and the DAT availability does not influence the P300 ERP amplitude or latency. P300 ERP amplitude reduction could be an indicator of the progression of illness and chronicity.

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Key words: Dopamine transporter, ERP, medication-naïve schizophrenia, meta-analysis, P300 waveform, single photon emission computerized tomography.

Introduction

The P300 wave is an event-related potential (ERP) that is recorded as a positive deflection on an electroencephalogram (EEG) about 300 ms after a participant receives an attended unusual or task-relevant stimulus. It is typically elicited by infrequent sensory stimuli in a

row of repeated stimulus presentations (Polich, 1998; Duncan *et al.* 2009) and reflects an endogenous cognitive process triggered in analyzing sensory stimuli (Picton, 1992; Polich, 2007).

The P300 amplitude is interpreted as a correlate of resources allocated to processing the task-relevant stimulus (Curran, 2004; Azizian & Polich, 2007). The origin of the P300 involves the complex summation of activity from multiple brain regions, particularly the various association areas of the cerebral cortex and the limbic system (Picton, 1992; Bledowski *et al.* 2004; Polich, 2007; Mangalathu-Arumana *et al.* 2012). However, little is known about the neurochemical

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substrates of the P300 and the specific neurotransmitter systems involved in generating this ERP response (Picton, 1992; Frodl-Bauch *et al.* 1999; Nieuwenhuis *et al.* 2005; Polich & Criado, 2006).

Patients with schizophrenia have been reported to have P300 amplitude reduction and latency prolongation compared to healthy controls (Frangou *et al.* 1997; Coburn *et al.* 1998; Ozgurdal *et al.* 2008; Shin *et al.* 2010; Jahshan *et al.* 2013). Neurophysiological changes, such as a reduction in P300 amplitude, have been described in populations at ultra-high risk (UHR) of developing schizophrenia (van der Stelt *et al.* 2005; Bramon *et al.* 2008; Frommann *et al.* 2008; van Tricht *et al.* 2010) and those changes may run a progressive course, from the prodromal to the chronic phases of schizophrenia (Ozgurdal *et al.* 2008). However, a longitudinal study has shown that these impairments remain stable from the prodrome up to the development of a full psychotic episode (van Tricht *et al.* 2011). Two meta-analyses of P300 in auditory oddball paradigms found a large pooled standardized effect size (PSES) of 0.85 and 0.89 respectively for P300 amplitude differences between patients with schizophrenia and controls at central (Cz) and parietal (Pz) midline electrodes (Jeon & Polich, 2003; Bramon *et al.* 2004). The majority of studies, however, involved patients treated with various antipsychotic drugs and those reports of unmedicated cases were not necessarily antipsychotic naive (Bramon *et al.* 2004).

Although antipsychotic drugs have been found to increase the P300 amplitude in some studies (Mathalon *et al.* 2000a; Bramon *et al.* 2004; Molina *et al.* 2004), it is generally not restored to normal levels (Ford *et al.* 1994; Hirayasu *et al.* 1998; Jeon & Polich, 2003). Family studies suggest that the unaffected and unmedicated relatives of patients may show similar, albeit milder, deficits (Bramon *et al.* 2005). Therefore, P300 measures in auditory oddball paradigms are thought to be vulnerability indicators for schizophrenia. Nevertheless, the influence of antipsychotics on ERP measures is poorly understood and medication confounding remains a possibility.

The dopamine transporter (DAT) is located primarily on the presynaptic membrane of dopaminergic neurons and plays a crucial role in the regulation of dopamine concentration in the synaptic cleft by dopamine reuptake. The involvement of dopaminergic mechanisms in the generation of the P300 is not yet clear (Kenemans & Kahkonen, 2011), especially because dopamine modulates the activity of the cortical networks through interaction with other neurotransmitters (Peters *et al.* 2004; Ford *et al.* 2007). However, the loudness dependence of the auditory evoked potential (LDAEP) was reported to be positively associated with DAT (Lee *et al.* 2011). D₂

antagonists may affect ERP latencies and amplitudes in healthy subjects (Takeshita & Ogura, 1994) and significant correlations of P300 parameters and striatal dopamine D₂/D₃ receptor availability have been reported (Pogarell *et al.* 2011).

The aims of our study were to explore ERP differences between drug-naïve patients with schizophrenia and healthy controls and to examine if P300 performance is related to DAT availability, both without the confounding effects of medication. This was made possible because a subset of the participants in this study also volunteered to undergo single photon emission computerized tomography (SPECT) with [^{99m}Tc]-TRODAT-1. In view of previous studies (Bramon *et al.* 2004; Chen *et al.* 2013), we predicted that the P300 amplitude would correlate positively with DAT availability whereas latency would have a negative correlation with DAT availability in patients with schizophrenia.

Method

Sample

All study participants were living in Tainan City, the fifth largest city in Taiwan with a population of 1 873 579. A total of 36 drug-naïve first-episode patients with schizophrenia were recruited at the psychiatric out-patient clinic of the National Cheng Kung University Hospital. One hundred and thirty-eight healthy community residents of Tainan City were also recruited through advertisements. These controls were interviewed by senior psychiatrists who had been practicing for more than 10 years, using the Chinese version of the Mini International Neuropsychiatric Interview (MINI; Sheehan *et al.* 1998) to ensure that they were free of any Axis I or Axis II psychiatric diagnoses. Brain magnetic resonance imaging (MRI) scans in the controls and the patients were normal. Among the patients, the mean duration of illness was 34.4 months (S.D.=66.2, median=7.9, interquartile range=21.7).

Before any procedure was performed, written informed consent was obtained from all participants after a complete explanation of the study. The Ethical Committee for Human Research at the National Cheng Kung University Hospital approved the study protocol.

Inclusion criteria were as follows: (1) patients should fulfill DSM-IV criteria for schizophrenia; (2) all participants aged between 18 and 60 years; (3) controls never received any antipsychotic treatment and were free of any psychotropic medication at the time of testing. The patients had never had any psychotropic medications prescribed and our clinic was their first contact

with psychiatric services. Exclusion criteria for all participants were: (1) other co-morbid psychiatric illnesses, substance abuse/dependence or neurological illnesses; (2) physical illness or unstable vital signs, or history of substance abuse/dependence as assessed during the clinical interview with the research psychiatrist, at the time of enrollment; (3) mental retardation; (4) all female participants of child-bearing age had to take an acceptable form of contraceptive throughout the duration of the study to be included; they also underwent an instant urine pregnancy test prior to starting the experiment; (5) all patients who were deemed at risk of acute suicide/self-harm were excluded from the study for their safety.

ERP data acquisition and analysis

A two-tone auditory P300 auditory oddball task was used for collecting EEG data. Stimuli were 80-dB tones with a 2-s interstimulus interval presented through bilateral intra-aural earphones; 80% of the tones were 'non-targets' of 1000 Hz and 20% were 'targets' of 2000 Hz in a random sequence. Subjects were instructed to press a button in response to targets only (Frangou *et al.* 1997). EEG data were collected from three midline scalp sites [frontal (Fz), central (Cz) and parietal (Pz)] according to the 10/20 International System (Jasper, 1958) using a 32-channel Quik-Cap with silver/silver-chloride sintered electrodes (Compumedics Neuroscan, USA). FPz was the ground, linked bilateral mastoids were the reference and a vertical bipolar channel with electrodes placed above and below the left eye was used to monitor eye blinks and eye movements. Data were digitized continuously at 500 Hz with a 0.1–40-Hz bandpass filter (24 dB/octave roll-off). Impedances were kept below 5 k Ω . Epochs from 100 to 600 ms pre- and post-stimulus respectively were averaged separately for target and non-target tones. Only epochs with correctly detected targets and correctly ignored non-targets were included in the averages. Epochs with evidence of eye blinks (± 50 μ V) were automatically rejected, as were those showing movement or other artifacts in any of the 32 channels. The P300 was defined as a positive waveform generated by the target tones and peaking between 200 and 400 ms post-stimulus. Its peak amplitude (measured with respect to the baseline) and its peak latency (measured from time zero) were both calculated using a computer algorithm that made the process blind to clinical group status (Bramon *et al.* 2006, 2008).

[^{99m}Tc]-TRODAT-1 SPECT and MRI

Each subject received a bolus intravenous injection of 740 MBq (20 mCi) of [^{99m}Tc]-TRODAT-1 (Institute of

Nuclear Energy Research, Taiwan) in a quiet environment approximately 10 min after the intravenous line was set up. The brain SPECT images were acquired 4 h after the injection. To avoid tilt and misalignment of the participant's head, we carefully positioned participants and monitored them during scanning, and used a head holder to further reduce movement artifacts. Before image acquisition, the participant was informed of the necessity to avoid head movement. Sinograms were reviewed blind to diagnosis to determine whether post-acquisition correction for head movements was needed. Movement correction was conducted with the motion correction software ICON version 8.5 KB21 (Siemens, USA).

We used a triple-headed rotating gamma camera (Multispect 3; Siemens, USA) with ultra high-resolution fan-beam collimators, which yields an image resolution of approximately 8.5 mm for the full-width at half-maximum (FWHM). The SPECT images were acquired over a circular 360° rotation, with 120 steps, at a rate of 50 s/step, in a 128×128×16 matrix. The images were then reconstructed using Butterworth and Ramp filters (Friston *et al.* 1990) (cut-off frequency=0.3 Nyquist, power factor=8), with attenuation according to Chang (1978). The reconstructed transverse images were realigned parallel to the canthomeatal line. The slice thickness of each transverse image was 2.89 mm. For semiquantitative analyses, six consecutive transverse slices on which the highest striatum uptake was best visualized were combined to obtain a slice of thickness 17.34 mm. Then regions of interest (ROIs) were placed over the striatum and the occipital cortex. The ROIs were drawn directly on the SPECT images by an experienced nuclear medicine physician who was blind to the participants' clinical data. The participants' MRI scans (GE, SIGNA CV-I, 1.5 T, USA), obtained within 2 weeks after SPECT examination, were used as a visual reference to determine the areas of the ROIs. The sizes of all ROIs were at least twice that of the FWHM. The specific striatal [^{99m}Tc]-TRODAT-1 binding (which represents striatal DAT availability) was calculated as the mean count of ROIs in the striatum divided by the mean count of ROIs in the occipital region (St/Oc) (Hwang *et al.* 2004).

Psychopathology ratings

On the day of recruitment, standardized psychopathology ratings were collected for all patients using the Clinical Global Impression Severity of Illness (CGI-S; Guy, 1976) and the Structured Clinical Interview for the Positive and Negative Syndrome Scale (SCI-PANSS; range 30–210 from least to most symptomatic) (Kay *et al.* 1987).

Statistical analyses

We performed a sample size calculation by a power analysis, which indicated that 40 patients and 160 controls would yield an 80% chance (power=0.8) of detecting an effect size (ES) of 0.5 between two groups using an independent-sample *t* test (two-tailed $\alpha=0.05$). Demographic differences between patients and controls were examined with χ^2 tests for categorical variables or with the Student *t* test for continuous variables. For the latter, Levene's test was used to assess the assumption of equality of variances. Diagnostic plots and one-sample Kolmogorov–Smirnov tests were used to test for normality.

Local study

The first aim of our study was to assess the differences between antipsychotic-naïve patients with schizophrenia and healthy controls in the P300 ERP. Mixed modeling was used to compare P300 amplitude or latency at three midline scalp sites (Fz, Cz and Pz) between the two clinical groups. The models included subject-varying intercepts to acknowledge the correlations between the three repeated measures for each participant. To assess whether group differences varied between the three sites, we tested whether a group \times electrode interaction was evident. Based on the previous literature we considered age and gender to be potential confounders of the group effect on P300 measures and therefore adjusted our analyses by including age and gender as covariates in our analysis model (Jeon & Polich, 2003; Wang *et al.* 2003; Yu *et al.* 2005). To evaluate the effects of aging and gender on the P300 amplitude and latency, we present the regression coefficients obtained from the above-described model. We used residual diagnostics to determine the shape of the relationship between age and P300 latency or amplitude, comparing a model for a quadratic relationship with the simpler linear relationship model. We further expanded this model to check whether group effects varied with age or gender by testing respective interaction terms. Where interaction terms were not significant, they were dropped from the final model.

The second aim of this study was to investigate a possible relationship between P300 amplitude or latency and DAT availability measured by SPECT with [^{99m}Tc]-TRODAT-1. Thus, for the subset of participants who provided DAT availability measures, we expanded the final model described above, adding the mean TRODAT binding ratio as an explanatory variable whose effect is of interest. As in our recent study and others (Hwang *et al.* 2004; Chen *et al.* 2013), the specific striatal [^{99m}Tc]-TRODAT-1 binding was calculated as St/Oc. The expanded analysis assumes that missingness of the TRODAT binding ratio

was driven only by explanatory variables of the model (age, gender, and group).

Statistical significance was established at $p < 0.05$. SPSS version 16 (SPSS Inc., USA) was used for all analyses.

Meta-analysis

We conducted a random effects meta-analysis combining the published literature and the local study presented here. We searched the Institute for Scientific Information Web of Knowledge, Scopus and PubMed (U.S. National Library of Medicine, NLM) using the following key words: 'drug-naïve', 'schizophrenia', 'event-related potential' and 'P300'. The search covered the period between 1950 and the beginning of 2012 and yielded a total of 16 articles with English abstracts. Of these, we excluded one review, four studies with visual ERP, including one with stroboscopic stimuli, two studies with mixed medicated and drug-naïve patients compared to controls, one pharmaceutical study without patient recruitment, one study with correlations only reported between P300 and brain CT imaging, and two conference abstracts. Thus, the meta-analysis included five articles published between 1998 and 2010 that focused on drug-naïve schizophrenia patients using the auditory oddball paradigm P300 ERP. Two of the five articles (Hirayasu *et al.* 1998; Wang *et al.* 2005) did not offer the mean and standard deviation values of the latency and amplitude, so finally only three articles (Wang *et al.* 2003, 2010; Xiong *et al.* 2010) were included in our meta-analysis. The standardized mean difference between controls and patients was computed for each primary study as Cohen's *d* (Cohen, 1988). The meta-analysis was conducted using the metan routine available in Stata version 10 (Stata Corporation, USA).

Results

Local study

The sample included 36 drug-naïve patients with a DSM-IV diagnosis of schizophrenia or schizophreniform psychosis along with 138 healthy controls. All the patients were presenting to services for the first time. The demographic characteristics of the patient and control groups are summarized in Table 1. Patients and controls had a similar gender distribution and smoking habits, with only a minority of participants, 10% of patients and 23% of controls, being smokers. However, compared to the controls, the patients were significantly younger ($t=4.56$, $df=95.4$, $p<0.001$), had spent significantly less time in education ($t=3.32$, $df=171$, $p=0.001$) and were less likely to live with a partner ($t=7.38$, $df=1$, $p=0.007$). Of the 36 patients

Table 1. Demographic characteristics of patients with schizophrenia ($n=36$) and controls ($n=138$)

	Patients	Controls	Statistical test		
			t/χ^2	df	p
Age (years)	24.7 (5.8) 18–39	30.6 (10.0) 19–58	4.56	95.42	< 0.001
Sex (male/female)	21/15	74/64	0.26	1	0.61
Smoking (yes/no)	3/29	25/109	1.59	1	0.21
Marital status (M/S) ^a	3/33	42/95	7.38	1	0.007
Years of education	13.3 (2.9) 5–21	15.0 (2.7) 6–24	3.32	171	0.001
Striatal DAT availability ^b	2.21 (0.23) 1.79–2.66 ($n=21$)	2.26 (0.21) 1.79–2.71 ($n=95$)	0.86	114	0.39
Duration of illness (months)	34.4 (66.2) 1.0–288.8	N.A.	N.A.	N.A.	N.A.
PANSS ($n=33$)					
Positive	21.4 (5.1) 8–30	N.A.	N.A.	N.A.	N.A.
Negative	18.9 (7.4) 8–43	N.A.	N.A.	N.A.	N.A.
General psychopathology	36.7 (13.5) 16–78	N.A.	N.A.	N.A.	N.A.
Sum	76.9 (23.0) 32–143	N.A.	N.A.	N.A.	N.A.
CGI-S ($n=25$)	4.7 (1.1) 2–6	N.A.	N.A.	N.A.	N.A.

DAT, Dopamine transporter; PANSS, Positive and Negative Syndrome Scale; CGI, Clinical Global Impression Severity of Illness; N.A., not applicable; df, degrees of freedom.

^a M includes married and living with a partner; S includes single, divorced, married but separated.

^b The specific striatal [^{99m}Tc]-TRODAT-1 binding as the measure of DAT availability was calculated as the mean count of regions of interest (ROIs) in the striatum divided by the mean count of ROIs in the occipital region (St/Oc).

Values given as number or mean (standard deviation) range.

included, 33 completed the PANSS and 25 completed the CGI-S (Table 1).

Group comparisons on P300 performance

The P300 ERP latency and amplitude of both patients and controls were normally distributed (non-significant Kolmogorov–Smirnov test and diagnostic plots showed no departure from normality). The group averages are shown in Fig. 1. The group \times electrode interactions were not significant (latency: $F=0.46$, $df=2,343$, $p=0.63$; amplitude: $F=1.97$, $df=2,343$, $p=0.14$) and were therefore dropped from the model.

After controlling for age and gender, the P300 ERP showed no significant difference between patients and controls in latency [estimated group difference = -3.85 ms, 95% confidence interval (CI) -15.60 to 7.91 ms; $F=0.42$, $df=1,170$, $p=0.52$] or in amplitude (estimated group difference = 0.27 μ V, 95% CI -1.14 to 1.68 μ V; $F=0.14$, $df=1,170$, $p=0.71$), as in Table 2.

In the same model, there was no effect of gender on P300 latency (estimated difference = 1.34 ms, 95% CI -7.93 to 10.60 ms; $F=0.81$, $df=1,170$, $p=0.78$). However, there was a significant effect on P300 amplitude, which was lower in males than in females (estimated difference = -1.49 μ V, 95% CI -2.60 to -3.78 μ V; $F=6.99$, $df=1,170$, $p=0.01$). Similarly, we did not observe any significant effects of age on P300 latency (estimated difference = -0.19 ms/year, 95% CI -0.69

to 0.31 ms/year; $F=0.56$, $df=1,170$, $p=0.46$) or on P300 amplitude (estimated difference = -0.04 μ V/year, 95% CI -0.098 to 0.02 μ V/year; $F=1.58$, $df=1,170$, $p=0.21$). In addition, with regard to the effects of age, the residual diagnostics showed no evidence of departure from a linear effect and the quadratic effect of age was not significant (latency: $p=0.39$; amplitude: $p=0.43$), thus the simpler linear age model was used.

The interactions between group and sex on both P300 measures were not significant and were removed from the final model (latency: $F=1.01$, $df=1,169$, $p=0.32$; amplitude: $F=0.04$, $df=1,169$, $p=0.95$). Similarly, the effect of group \times age interaction on P300 amplitude was not significant ($F=2.70$, $df=1,169$, $p=0.10$). Of note, there was a significant effect of age \times group interaction on P300 latency (estimated difference = 1.51 ms, 95% CI -0.24 to 3.27 ms; $F=3.99$, $df=1,169$, $p=0.048$), with the difference between patients and controls in P300 latency became more pronounced with aging, and with latency increasing faster with age in the patient group (Fig. 2).

Relationships between P300 measures and DAT availability

The mean striatal DAT availability measures as determined by SPECT with [^{99m}Tc]-TRODAT-1 are shown in Table 1 for patients and controls. Having adjusted for age and gender in the same model, we found no effect of DAT availability on P300 latency ($F=1.30$,

Table 2. Latency/amplitude of patients with schizophrenia ($n=36$) and controls ($n=138$)

	Parietal	Central	Frontal	Statistical analysis	
				Coefficient	<i>p</i> value
Latency (ms)					
Schizophrenia	338.86 (29.24)	335.03 (31.21)	335.69 (32.61)	−3.85	0.52
Normal control	334.68 (32.52)	331.16 (32.79)	328.68 (33.77)		
Amplitude (μ V)					
Schizophrenia	8.09 (4.20)	7.63 (4.00)	6.31 (3.32)	0.27	0.71
Normal control	8.00 (4.04)	7.32 (4.29)	7.08 (4.42)		

Age, sex and electrode site controlled for statistical analyses.
Values given as mean (standard deviation).

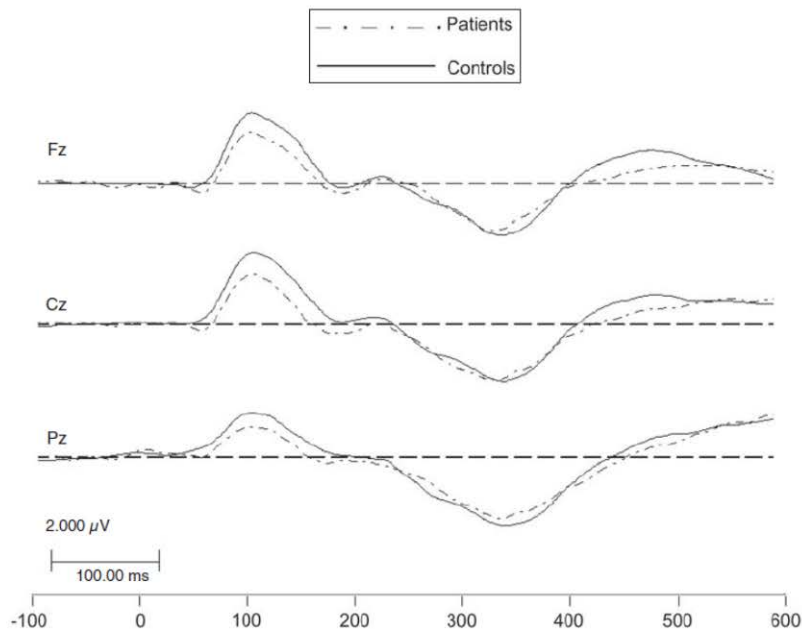


Fig. 1. P300 event-related potential (ERP) group averages. An eliciting event is presented repeatedly and the resulting P300 ERPs (aligned at the time point of the eliciting event) are averaged to yield the subject's average P300 ERP on different sites (Fz, Cz, Pz), in which activity not time-locked to the eliciting event averages out (i.e. to zero) and what remains is the time-locked wave series. P300 is a positive-going wave with a scalp amplitude distribution in which it is largest parietally (Pz) and smallest frontally (Fz), taking intermediate values centrally (Cz).

$df=1,111$, $p=0.26$) or amplitude ($F=1.28$, $df=1,111$, $p=0.26$).

Meta-analysis

We conducted a random effects meta-analysis of the available literature of drug-naïve patients with the

auditory oddball paradigm P300 ERP between 1950 and 2012, including three previously published articles and our current data. The combined sample included 105 drug-naïve patients with schizophrenia and 214 healthy controls. The latency PSES computed as Cohen's d was -0.13 (95% CI -0.37 to 0.12 , $p=0.31$), indicating that patients and controls did not

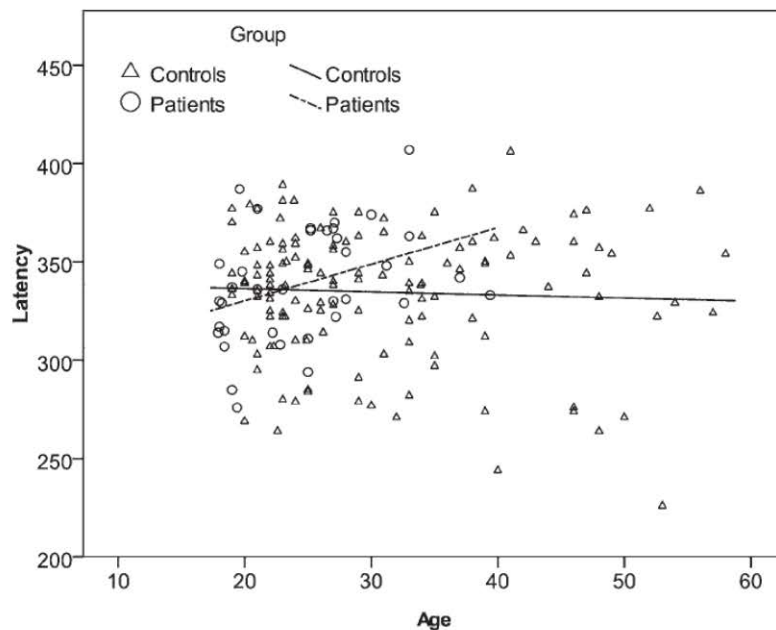


Fig. 2. A significant effect of age \times group interaction was seen on parietal P300 latency, with the difference between patients and controls in P300 latency becoming more pronounced with aging and with latency increasing faster in the patients. The relationship between latency and age was similar at the other two sites (central and frontal).

differ significantly. There was no significant between-study heterogeneity in ES (coefficient = -0.13 , $p = 0.38$). The amplitude PSES size, again computed as Cohen's d , was 0.48 (95% CI -0.002 to 0.97 , $p = 0.05$), with only patients showing a significant reduction in amplitude. There was no significant between-study heterogeneity in ES (coefficient = 0.48 , $p = 0.14$). Fig. 3 shows the forest plots for the latency and amplitude meta-analyses.

Discussion

Our study shows that the medication-naïve patients with schizophrenia did not differ significantly from the healthy controls in measures of the P300 ERP. Furthermore, in our sample we found no evidence that DAT availability influenced P300 amplitude or latency. The strength of our study is that we recruited a relatively large and clinically homogeneous sample, all the subjects were drug naïve and patients were at a uniform and early stage of their illness.

We suggest that our failure to find P300 amplitude or latency differences between drug-naïve patients and normal controls might be related to the relatively short duration of psychotic illness of our patients. Indeed, previous studies have identified a correlation

whereby the P300 amplitude was more reduced and latency more delayed with longer illness duration (Mathalon *et al.* 2000b). The P300 ERP involves the complex summation of activity from multiple brain regions, including the various association areas of the cerebral cortex and the limbic system (Picton, 1992; Bledowski *et al.* 2004; Polich, 2007; Mangalathu-Arumana *et al.* 2012), which in turn are thought to originate from deeper brain sources such as the striatum (Kellendonk *et al.* 2006; Howes & Kapur, 2009). The lack of correlation between P300 performance and striatal DAT availability in the current study is in line with our previous findings that DAT availability is not impaired in drug-naïve patients with schizophrenia (Chen *et al.* 2013) and with the progressive brain pathophysiological process correlating with duration of illness as mentioned (Mathalon *et al.* 2000b).

There was a significant gender effect in our combined sample, with the P300 amplitude being lower in males than females, as reported in a previous study on healthy individuals (Schiff *et al.* 2008). This is consistent with the later age of onset and lower incidence of schizophrenia in females (Faraone *et al.* 1994).

Previous studies have reported that the unaffected relatives of patients and other populations at risk for psychosis have similar, but milder, P300 amplitude

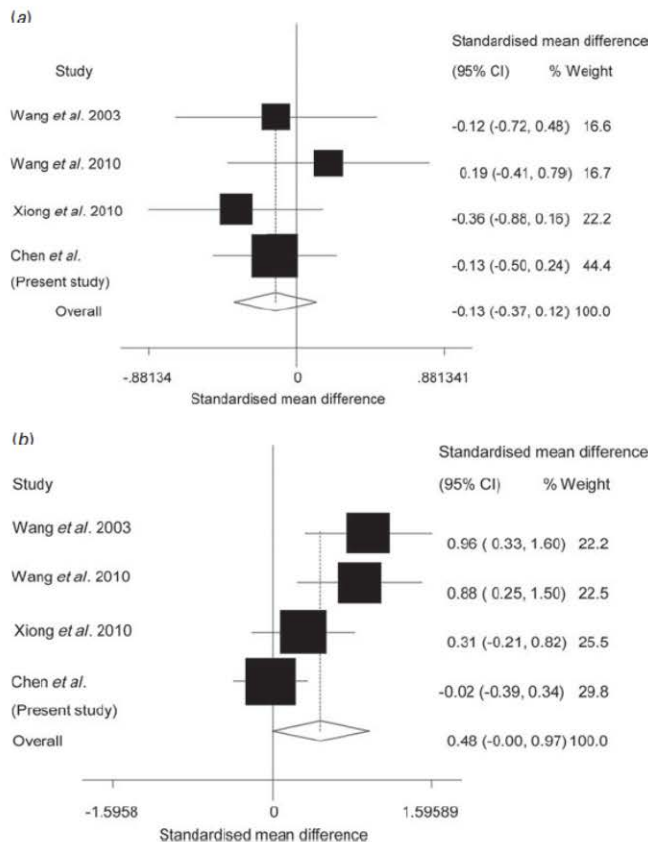


Fig. 3. Meta-analysis forest plots of (a) P300 latency and (b) P300 amplitude, showing the standardized effect sizes (SES) for each study. The horizontal lines represent 95% confidence intervals (CIs) for the SES in each study. The size of the squares represents the weights given to the studies. The diamond shows the pooled standardized effect size (PSES) of all studies using meta-analysis tools in Stata 10.

and latency deficits, suggesting that the P300 amplitude reductions and latency delays may be conceptualized as biomarkers of genetic predisposition to psychosis (van Beijsterveldt & van Baal, 2002; Bramon *et al.* 2005, 2008). Our data challenge this notion and indicate that the P300 could instead be a marker of disease chronicity/progression.

Some studies have shown a P300 amplitude reduction in populations at UHR for psychosis (Yung *et al.* 2005) either prior to the onset of psychosis compared to controls (Bramon *et al.* 2008; Frommann *et al.* 2008) or after transition to psychosis compared to the state before transition (van Tricht *et al.* 2010). However, the basis of the vulnerability to psychosis is not fully understood (Fusar-Poli *et al.* 2013a,b). Hence, such patients could have a different underlying pathophysiology, compared with established schizophrenia,

that is separate from a genetic vulnerability to schizophrenia and may relate to early states of mixed psychopathology. It is also possible that some ERP components, such as P300 amplitude abnormalities, could be present before a psychotic episode but do not show further progression immediately following the psychotic onset (van Tricht *et al.* 2011), assuming of course that such individuals are not on antipsychotic medication, which cannot always be guaranteed. Therefore, our data suggest the P300 could instead be a marker of disease chronicity/progression in patients with schizophrenia but not in the UHR population. Nevertheless, the discrepancy between our data and results from UHR population ERP studies needs further exploration.

The effect of psychotropic medication on evoked potentials is controversial. P300 amplitude and latency

in patients with schizophrenia may tend to normalize after pharmacological intervention (Coburn *et al.* 1998; Gonul *et al.* 2003), but the absence of such drug effects has also been reported (de Wilde *et al.* 2008). Several studies conducted on unmedicated patients who were not drug naïve suggest that they also show amplitude reductions and latency delays (Bramon *et al.* 2004). Our findings of normal P300 performance in medication-naïve patients would support an enduring confounding effect of medication; however, longitudinal studies before and after introducing medication are required to clarify this matter.

The meta-analysis including our data found that drug-naïve patients with schizophrenia do not have significant impairments in P300 latency compared with controls. Considering the pooled ES of -0.13 and the range of ESs included in the 95% CI, the SES is unlikely to exceed -0.37 , which is conventionally considered to be a moderate effect (Cohen, 1988). Therefore, our data and the meta-analysis of the literature show fairly convincingly that, in medication-naïve patients, the P300 latency is unlikely to be impaired.

The meta-analysis of P300 amplitude yielded a much larger PSES of 0.48 with a trend for significance. Considering all the evidence, we are inclined to conclude that there are probably no clinically relevant impairments in amplitude in drug-naïve patients in the early stages of the illness. We excluded studies of drug-free previously treated patients that were reported as showing P300 differences between patients and controls; hence their inclusion may have changed the outcome of our meta-analysis. Unfortunately, there were too few studies eligible for inclusion for us to examine publication bias and heterogeneity in a meaningful way.

We found a significant effect of age \times group interaction on P300 latency in our data, whereby the patients showed greater latency delays with aging; thus the difference between patients and controls in P300 latency became more pronounced with increasing age (Gilmore, 1995; O'Donnell *et al.* 1995; Wang *et al.* 2003; Araki *et al.* 2006). This finding is not surprising and could reflect a progressive neurodegenerative change, a faster age-related decline in the speed of neural transmission among patients with schizophrenia.

A potential limitation is that patients recruited into our study had to be relatively stable and well enough to complete an extensive battery of clinical, cognitive, EEG and neuroimaging tests, which may potentially reduce the generalizability of our findings to a wider population including more severely ill and/or less cooperative patients. Furthermore, a study of only non-smokers would not be representative of most clinical populations, and we included participants regardless

of their smoking habit. Our patient and control groups were not significantly different in smoking status; hence, smoking is unlikely to have confounded our results. Our controls were recruited from the local community through research advertisements and, compared to the patients, they were significantly older, had spent more time in education and were more likely to be living with a partner; these demographic differences were comparable to previous studies (Loughland *et al.* 2010). We adjusted all our analyses by age and gender; thus we consider that our controls provided a suitable comparison group and were representative of the local healthy population. Family studies suggest that the unaffected and thus unmedicated relatives of patients may show similar, albeit milder, deficits in the P300 as schizophrenia patients. As our controls were not screened against having a family history of psychosis, this might potentially confound the results and contribute to a smaller difference between patient and control groups.

Our findings suggest that the P300 ERP is not altered in the early stages of schizophrenia before medication is introduced, and that the DAT availability does not influence the P300 ERP amplitude or latency. The P300 ERP could be an indicator of the progression of illness and chronicity. We identified gender differences and aging effects that could have clinical significance and may be taken into account in future studies. As there are only four P300 ERP studies, including this one, of drug-naïve patients with schizophrenia, all of them cross-sectional, further longitudinal studies are needed to explore the effects of medication and duration of illness on the P300 ERP.

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Declaration of interest

None.

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Chapter 4

Overall discussion and future directions

This thesis aims to investigate drug naïve patients with schizophrenia and examine their DAT availability using SPECT and their P300 ERP performance. My colleagues and I also investigated how DAT availability and P300 measures related to their psychopathology ratings. We explored the relationship between DAT availability and P300 performance. In the thesis I presented our group's own novel data and I conducted meta-analyses of DAT availability and P300 performance of all available literature.

(4.1) Summary of Thesis Findings

My findings show that the specific striatal binding ratio of drug naïve patients with schizophrenia was not significantly different to that of normal controls, indicating that both groups had similar dopamine transporter (DAT) availability. My DAT study is the largest to date, including over twice the sample of drug naïve/drug free patients of the next largest study of DAT in schizophrenia (Laruelle *et al.*, 2000). Another strength of my study is that all the subjects were drug-naïve, and at a uniform and early stage of their illness. My findings are consistent with previous SPECT studies of schizophrenia using [^{99m}Tc]-TRODAT-1, which were included in my meta-analysis (Hsiao *et al.*, 2003; Yang

et al., 2004; Schmitt *et al.*, 2005; Schmitt *et al.*, 2006; Schmitt *et al.*, 2008; Chou, 2010).

The DAT meta-analysis including my data found there was no evidence for a difference in striatal [^{99m}Tc]-TRODAT-1 binding in drug-naïve patients with schizophrenia compared to controls. Considering the range of effect sizes included in the 95% confidence interval, the standardized effect size is unlikely to exceed 0.31, which is conventionally considered to be a small effect (Cohen, 1988).

My other findings show that drug naïve patients with schizophrenia did not differ significantly from healthy controls in amplitude or latency of the P300 ERP. Furthermore, in my sample I found no evidence that DAT availability influences P300 amplitude or latency.

The main strengths of my P300 ERP study are again that I recruited a large and clinically homogenous sample, all the subjects were drug naïve and patients were at a uniform and early stage of their illness. Thus compared to most previous studies, mine can avoid the confounding effects of medication and chronicity of illness.

The P300 meta-analysis including my data found that, drug-naïve patients with schizophrenia do not have significant impairments in P300 latency compared with controls. Considering the pooled effect size of -0.13 and the range of effect sizes included in the 95% CI, the standardized effect size is unlikely to exceed -0.37, which is conventionally considered to be a moderate effect (Cohen, 1988). The meta-analysis of P300 amplitude yielded a much larger pooled standardized effect size of 0.48 with patients showing a trend for significance in amplitude reductions (Cohen, 1988).

(4.2) DAT Availability: Methodological Considerations

It is important to consider the possible influence of tobacco smoking since a decrease in DAT availability in the striatum of smokers has been described in previous studies (Newberg *et al.*, 2007). A study of non-smokers only would not be representative of most clinical populations with psychotic disorders and I included participants regardless of their smoking habit. My patient and control groups were not significantly different in smoking status; hence smoking is unlikely to have confounded my results. Nevertheless, all my analyses were adjusted for tobacco smoking in this sample.

One potential limitation of my study is that the regions of interest (ROIs) were manually drawn directly on the SPECT image. Whilst there is the potential for observer bias using this approach, I minimised this risk by ensuring that the ROIs were delineated by an experienced nuclear-medicine physician independent of the clinical assessment and blind to the subject's clinical group. We delineated the ROI on the SPECT image rather than on the MRI image as this does not require the ROI to be transformed from MRI to SPECT space. However, studies have found similar results using both approaches (Wang *et al.*, 1996; Inoue *et al.*, 2004) indicating that the choice of approach is unlikely to have influenced our findings.

Another potential limitation is that patients recruited in my study for SPECT imaging had to be cooperative and relatively stable in order to complete the procedure. This is an issue for most imaging studies and may potentially affect the generalisability of my findings to the most severely ill and/or uncooperative patients.

(4.3) Interpretation of Thesis Findings

(i) DAT Availability

The pre-synaptic DAT plays a key role in regulating the dopamine (DA) content in the synaptic cleft by transporting it back into DA terminals, effectively modulating the concentration of DA available for postsynaptic receptor binding (Bannon *et al.*, 2001). There are two main interpretations of my findings: on the one hand, if DA was able to displace [^{99m}Tc]-TRODAT-1 from DAT, my failure to find a difference in specific binding could be due to elevated synaptic DA levels in schizophrenia masking an increase in DAT levels (Abi-Dargham *et al.*, 2000). However, whilst the definitive studies have not yet been conducted, the available evidence indicates that [^{99m}Tc]-TRODAT-1 binding is not sensitive to variations in DA levels seen *in vivo* (Fernagut *et al.*). A more likely interpretation is thus that DAT levels are unaltered in schizophrenia. This is important as it indicates that there is no compensatory increase in DAT levels in response to the increased release and synaptic levels of DA previously reported in schizophrenia (Laruelle *et al.*, 1999; Abi-Dargham *et al.*, 2000). This interpretation is consistent with evidence that fluctuations in brain DA levels do not alter the abundance of the DAT (Moody *et al.*, 1996). My findings might thus suggest pharmacological augmentation of DAT as a potential novel treatment strategy for schizophrenia.

I identified a sex effect in the combined sample whereby females had a higher specific striatal radiotracer binding ratio than males, which is consistent with a previous study (Kuikka *et al.*, 1997). The effect size for sex in my study is 0.25 (Cohen's *d*) and although this is a small effect, it might be of relevance. It would seem to be in line with the later age of onset and lower incidence of schizophrenia

in females (Faraone *et al.*, 1994). The lower DAT availability in males could contribute to their earlier age of onset as, putatively, they would have less capacity to buffer excess striatal DA release.

My finding that there is a negative relationship between age and DAT availability is consistent with a previous study showing that the specific uptake of [^{99m}Tc]-TRODAT-1 radiotracer decreases with advancing age in healthy individuals (Mozley *et al.*, 1999). Since the density of striatal DA D₂/D₃ receptors also declines with age in healthy individuals (Chen *et al.*, 2005), my data indicate the consistency of this age effect on both pre- and post-synaptic dopaminergic function.

The PANSS scores for positive and negative symptoms of schizophrenia and general psychopathology did not correlate with the [^{99m}Tc]-TRODAT-1 binding ratio. This is again consistent with previous research (Hsiao *et al.*, 2003; Yang *et al.*, 2004; Schmitt *et al.*, 2005) and with the absence of alterations in [^{99m}Tc]-TRODAT-1 availability in my patients as a group.

The meta-analysis including my data found there was no evidence for a difference in striatal [^{99m}Tc]-TRODAT-1 binding in drug-naïve patients with schizophrenia compared to controls. Considering the range of effect sizes included in the 95% confidence interval, the standardized effect size is unlikely to exceed 0.31, which is conventionally considered to be a small effect (Cohen, 1988). With this effect size, as many as 188 drug-naïve patients and an equal number of controls would need to be tested to have 85% power to detect a difference (two-group T test with a 5% two sided significance level). The overall conclusion of my work is that drug-naïve patients in the early stages of schizophrenia have normal [^{99m}Tc]-TRODAT-1 binding and that any impairments, if present, would be of a small magnitude. Given the normal variation in binding,

any potential deficits in drug-naïve patients are unlikely to be clinically significant. Although [^{99m}Tc]-TRODAT-1 binds to serotonin transporters, blocking studies show that the specific binding in the striatum is almost entirely due to dopamine transporters (Dresel *et al.*, 1999), hence, my findings reflect that DAT availability is unaltered in drug-naïve patients with schizophrenia.

(ii) **P300 ERP**

I suggest that our failure to find P300 amplitude or latency differences between drug naïve patients and normal controls might be related to the relatively short duration of psychotic illness of my patients. Indeed, previous studies have identified a correlation whereby the P300 amplitude was more reduced and latency was more delayed with longer illness duration (Mathalon *et al.*, 2000b). The P300 ERP involves the complex summation of activity from multiple brain regions, particularly the various association areas of the cerebral cortex and the limbic system (Picton, 1992; Bledowski *et al.*, 2004; Polich, 2007; Mangalathu-Arumana *et al.*, 2012), which in turn are thought to originate from deeper brain sources such as the striatum (Kellendonk *et al.*, 2006; Howes & Kapur, 2009). The lack of correlation between P300 performance and striatal DAT availability in this study is in line with my previous findings that DAT availability is not impaired in drug naïve patients with schizophrenia (Chen *et al.*, 2013) and with the progressive brain pathophysiological process correlating with duration of illness as mentioned above (Mathalon *et al.*, 2000b).

There was also a significant sex effect in my combined sample, whereby the P300 amplitude was lower in males compared to females, as reported in a previous study on healthy individuals (Schiff *et al.*, 2008). This is consistent with

the later age of onset and lower incidence of schizophrenia in females (Faraone *et al.*, 1994).

Previous studies have reported that the unaffected relatives of patients and other populations at risk for psychosis have similar, yet milder P300 amplitude and latency deficits, suggesting that the P300 amplitude reductions and latency delays may be conceptualized as biomarkers of genetic predisposition to psychosis (van Beijsterveldt, 2002; Bramon *et al.*, 2005; Bramon *et al.*, 2008). My data challenge this notion and indicate that the P300 could rather be a marker of disease chronicity/progression.

Some studies have shown P300 amplitude reduction in populations of ultra high risk (UHR) for psychosis (Yung *et al.*, 2005) either prior to the onset of psychosis compared to controls (Bramon *et al.*, 2008; Frommann *et al.*, 2008) or after transition to psychosis compared to the state before transition (van Tricht *et al.*, 2010). However, the basis of their vulnerability to psychosis is not fully understood (Fusar-Poli *et al.*, 2013a; Fusar-Poli *et al.*, 2013c). Hence, compared to patients with established schizophrenia, such high risk individuals could have a different underlying pathophysiology that is distinct from a genetic vulnerability to schizophrenia. It is also possible that some ERP components, such as P300 amplitude abnormalities could be present before a psychotic episode but do not show further progression immediately following the psychotic onset (van Tricht *et al.*, 2011). Therefore, my data suggest the P300 could rather be a marker of disease chronicity/progression in patients with schizophrenia but not in the UHR population. Nevertheless, the discrepancy between my data and results from UHR population ERP studies clearly needs further exploration.

The role of psychotropic medication on evoked potentials is controversial. P300 amplitude and latency in patients with schizophrenia may tend to normalize

after pharmacological intervention (Coburn *et al.*, 1998; Gonul *et al.*, 2003), however, the absence of such drug effects has also been reported (de Wilde *et al.*, 2008). Several studies conducted on un-medicated patients who were not drug naïve suggest that they also show amplitude reductions and latency delays (Bramon *et al.*, 2004b). My findings of normal P300 performance in medication naïve patients would support an enduring confounding effect of medication, however longitudinal studies before and after introducing medication are required to clarify this matter.

The meta-analysis including my data found that, drug-naïve patients with schizophrenia do not have significant impairments in P300 latency compared with controls. Considering the pooled effect size of -0.13 and the range of effect sizes included in the 95% CI, the standardized effect size is unlikely to exceed -0.37, which is conventionally considered to be a moderate effect (Cohen, 1988). Therefore, my data and the meta-analysis of the literature show quite convincingly that in medication naïve patients the P300 latency is unlikely to be impaired.

The meta-analysis of P300 amplitude yielded a much larger pooled standardized effect size of 0.48 with a trend for significance (Cohen, 1988). Considering all the evidence, I am inclined to conclude there probably are no clinically relevant impairments in amplitude in drug naïve patients in the early stages of the illness. I excluded studies of drug-free previously treated patients which were reported as showing P300 differences between patients and controls; hence their inclusion may have changed the outcome of my meta-analysis. Unfortunately there were too few studies eligible for inclusion for me to examine publication bias and heterogeneity in a meaningful way.

I found a significant age by group interaction on P300 latency in my data, whereby the patients showed greater latency delays with ageing; thus the difference between patients and controls in P300 latency became more pronounced with increasing age (Gilmore, 1995; O'Donnell *et al.*, 1995; Wang *et al.*, 2003; Araki *et al.*, 2006). This finding is not surprising and could reflect a faster age-related decline in the speed of neural transmission amongst patients with schizophrenia.

A potential limitation is that patients recruited into my study had to be relatively stable and well enough to complete an extensive battery of clinical, cognitive, EEG and neuroimaging tests, which may potentially reduce the generalizability of my findings to a wider population including more severely ill and/or less cooperative patients. Furthermore, a study of non-smokers only would not be representative of most clinical populations, and I included participants regardless of their smoking habit. My patient and control groups were not significantly different in smoking status; hence, smoking is unlikely to have confounded my results. My controls were recruited from the local community through research advertisements and, compared to the patients, they were significantly older, had spent more time in education and were more likely to live with a partner; these demographic differences being comparable to previous studies (Loughland *et al.*, 2010). Family studies suggest that the unaffected and thus un-medicated relatives of patients may show similar if milder deficits in the P300 as schizophrenia patients. Since my controls were not screened against having a family history of psychosis, this might potentially confound results and contribute to a lesser difference between patient and control groups. Finally it should be noted that I statistically adjusted all my analyses by age and sex, thus

all things considered, I believe that my controls provided a suitable comparison group.

(4.4) Alternative Explanations for our Negative Findings

As described in the introduction, several studies have reported that the unaffected relatives of patients and other populations at increased risk for psychosis (such as people with sub-clinical psychotic experiences) have similar, yet milder P300 amplitude and latency deficits (Yung *et al.*, 2005; Bramon *et al.*, 2008; Frommann *et al.*, 2008; van Tricht *et al.*, 2010). This body of research suggests that the P300 amplitude reductions and latency delays are biomarkers of genetic predisposition to psychosis (van Beijsterveldt, 2002; Bramon *et al.*, 2005; Bramon *et al.*, 2008). A recent study claims that P300 performance can improve the accuracy of clinical assessments aiming to identifying those “at risk” individuals who go on to develop a full blown psychotic episode (Nieman *et al.*, 2014). My results however challenge this notion and indicate that the P300 may in fact be a marker of disease progression and chronicity.

Typically these “at risk” populations have undergone EEG testing prior to starting any antipsychotic treatment and medication confounding is highly unlikely to be a problem. However, these “at risk” samples tend to be particularly heterogeneous and their vulnerability to psychosis can have multiple and diverse origins that remain poorly understood (Fusar-Poli *et al.*, 2013a; Fusar-Poli *et al.*, 2013c). A minority of people with “prodromal” or “at risk” mental states have a known family history of psychosis. The majority of these populations actually have no identified genetic risk factors but do display a wide range of mild or short-lived psychotic experiences.

A further caveat to highlight is that the “ultra-high risk/prodromal” studies tend to undertake EEG at baseline and then follow up participants for one or 2 years after disease onset. To establish if P300 deficits are markers of disease progression and chronicity we will need to cover substantially longer periods of follow up and most importantly need to undertake the baseline as well as new follow up EEG recordings after illness onset. Such studies are complex and have not yet been done in the psychosis field.

A final and crucial alternative explanation is related to statistical power limitations of my sample. As described in chapter 3 of this thesis, I conducted a meta-analysis including both my data as well as the few existing comparable studies in drug-naïve patients. I found that, drug-naïve patients with schizophrenia do not have significant impairments in P300 latency compared with controls. From my meta-analysis I would argue that the standardized effect size is unlikely to exceed -0.37, which is conventionally considered a moderate effect (Cohen, 1988). Given this moderate effect size, my sample of 36 patients and 138 controls, would have only 60% power to detect a difference in latency (ES=0.4, two-group T test with a 5% two-sided significance level would yield about 60% power). Although my data and the meta-analysis of the literature show that in medication naïve patients the P300 latency is not impaired, the possibility of a type II error needs to be considered.

The meta-analysis of P300 amplitude yielded a much larger pooled standardized effect size of 0.48 with a trend for significance and a potentially large effect size according to the 95% confidence interval (Cohen, 1988). Therefore, my sample of 36 patients and 138 controls had at least 72% power to detect this larger effect size. However the P300 amplitude literature showed evidence of publication bias and as a result the meta-analysis could over-estimate

the strength of the effect. Although unlikely, again a type II error for amplitude needs to be considered as an alternative explanation.

Given the power estimations given above, it is conceivable that drug naïve patients with psychosis may have true impairments in P300 amplitude and latency that were missed both by my study and by the limited existing literature in this particular patient group. This alternative explanation would also be consistent with those studies that have identified P300 impairments in pre-psychotic at-risk populations and that view the P300 as a trait marker of disease vulnerability (Bramon *et al.*, 2005; Bramon *et al.*, 2008).

The evidence that P300 changes with illness duration

Several studies conducted with patients with schizophrenia have shown that the P300 amplitude and latency performance declines (smaller amplitude; longer latency) with a longer duration of illness and that this effect is not simply explained by normal ageing. Indeed the P300 amplitude was inversely correlated with illness duration (Olichney *et al.*, 1998). Similarly P300 latency prolongation in schizophrenic patients with longer illness durations has also been reported (O'Donnell *et al.*, 1995; Frangou *et al.*, 1997; Olichney *et al.*, 1998). According to Mathalon the P300 amplitude was more reduced and latency was more delayed with longer illness duration in patients with schizophrenia (Mathalon *et al.*, 2000b). Among patients with schizophrenia, P300 amplitude correlated positively with age of onset and negatively with illness duration. P300 latency correlated positively with age in patients with schizophrenia and also tended to increase with age in controls. However, the slopes of the latency-age relationships were significantly greater in patients with schizophrenia than in controls indicating a faster decline with ageing amongst patients. Finally, the P300 latency correlated

positively with illness duration but showed no relationship to age of onset (O'Donnell *et al.*, 1995; Frangou *et al.*, 1997). These studies support my hypothesis that the P300 deficits are markers of disease progression.

Conclusions on the interpretation of the P300 findings

In conclusion, the lack of clear differences between the first episode drug naïve cases and controls in the current study could indicate that:

(a) much of the research literature on P300 and schizophrenia is confounded by antipsychotic medication, and/or the effect of illness on P300 is much less than commonly supposed and hence less readily detected in small samples; and

(b) the P300 may be considered a marker of disease progression/chronicity. However, the discrepancy between these findings and results from ultra-high risk population ERP studies clearly needs further exploration and it is not possible to resolve the issue here. It is plausible that the P300 deficits described might be a non-specific marker of predisposition of some individuals to psychosis (hence the ultra-high risk and family study findings) as well as a marker of disease progression. Indeed some research supports the notion of the P300 being both a trait and state marker (Turetsky *et al.*, 1998a; Turetsky *et al.*, 1998b; Turetsky *et al.*, 2000).

According to Mathalon, the P300 amplitude reduction reflects not only a trait-marker characteristic of schizophrenia, but can also fluctuate with clinical state (Mathalon *et al.*, 2000b). Reduced P300 amplitude was found in patients whose symptoms had been improved or largely remitted with medication use (Blackwood *et al.*, 1987; Coburn *et al.*, 1998; Mathalon *et al.*, 2000a; Pass *et al.*, 1980; St Clair *et al.*, 1989; Rao *et al.*, 1995; Turetsky *et al.*, 1998a). Reduced

P300 amplitude has also been reported in first-episode patients (Hirayasu *et al.*, 1998; Salisbury *et al.*, 1998) as well as in unaffected first-degree relatives of schizophrenia (Blackwood *et al.*, 1991; Kidogami *et al.*, 1991; Roxborough *et al.*, 1993; Frangou *et al.*, 1997).

Moreover, other studies also implicate P300 amplitude as a clinical-state marker in schizophrenia by showing associations with clinical symptoms (Pfefferbaum *et al.*, 1989; Eikmeier *et al.*, 1992; Egan *et al.*, 1994; Maeda *et al.*, 1996; Turetsky *et al.*, 1998b; Ford *et al.*, 1999; Mathalon *et al.*, 2000a) as well as showing sensitivity to clinical-state fluctuations over time (Maeda *et al.*, 1996; Turetsky *et al.*, 1998a; Mathalon *et al.*, 2000a) and to medication effects independent of clinical state (Coburn *et al.*, 1998; Umbricht *et al.*, 1998). Thus, as a marker of both state and trait, P300 amplitude is particularly suitable for tracking progressive neuropathological processes over the long-term illness course.

Finally, an important limitation of the current P300 literature in schizophrenia including the current study as well as my meta-analysis is that virtually all published studies are cross-sectional investigations of independent samples at different levels of risk and at different disease and treatment stages. As a result, reconciling such a heterogeneous body of cross-sectional work is very challenging. Longitudinal cohort studies investigating EEG markers from the prodrome and re-testing participants over several years after disease onset are needed. This would be the ultimate way to establish if the P300 is a trait marker of vulnerability, a marker of disease progression or a combination of both. Although the data I have presented in my thesis are the baseline assessments of my participants at the point of recruitment, my colleagues and I at Tainan's University Hospital have continued to follow up these patients over several years.

We hope to soon complete a longitudinal 5-year follow up study including a range of neuroimaging and biological testing of this cohort of patients.

(4.5) Conclusions and Future directions

My DAT availability findings, in the largest first-episode drug-naïve and healthy sample studied to date, suggest that striatal DAT availability is not altered in the early stages of schizophrenia before medication is introduced. Instead, I identified sex differences and confirmed ageing effects that could have clinical significance and may be taken into account in future studies.

Furthermore, my P300 findings also suggest that the P300 ERP is not altered in the early stages of schizophrenia before medication is introduced, and the DAT availability does not influence the P300 ERP amplitude or latency. The P300 ERP could be an indicator of the progression of illness and chronicity. I also identified sex differences and aging effects as DAT availability findings that could have clinical significance and may be taken into account in future studies. As there are only 4 P300 studies of drug naïve patients with schizophrenia and all of them are cross sectional, further longitudinal studies which explore the effect of medication and duration of illness upon the P300 ERP are needed.

The importance of taking into account medication effects is emphasized by this thesis as is the value of converging methodologies such as research on families and other ‘at risk’ populations and longitudinal cohort studies.

In future, there are other endophenotypes for psychosis that I would like to investigate in my sample of drug naïve patients with schizophrenia. These include EEG markers like the mismatch negativity (MMN), which is a pre-attentive measure of auditory processing well characterized in psychosis (Bramon *et al.*,

2004a; Umbricht & Krljes, 2005). Another interesting endophenotype is the P50 waveform, which tackles brain gating mechanisms that are impaired in psychosis and could be useful endophenotypes for genetic research (Shaikh *et al.*, 2015). Magnetic Resonance Imaging (MRI) also offers a promising method to identify putative endophenotypes for psychotic disorders (McDonald *et al.*, 2004; Arnone *et al.*, 2009; van Erp *et al.*, 2015).

In a previous paper, we described how striatal dopamine D_{2/3} receptor availability, as measured by SPECT ligand [¹²³I] iodobenzamide (IBZM), was associated with schizotypal traits in healthy people (Chen *et al.*, 2012). Therefore we conducted a similar IBZM experiment with my drug naïve patient sample and this is the focus of another ongoing manuscript currently in preparation.

Further longitudinal follow up of the drug naïve patients participating in my research has already been initiated. These patients have agreed to attend regular post-treatment assessments, which might help us to answer the questions raised by this current study in the near future.

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APPENDIX 1

FURTHER DEMOGRAPHIC DATA

In chapters 2 and 3 of the thesis (published papers) only basic demographics were provided. Here we have included additional information on family history, age of onset/duration of illness and detailed psychopathology data. Although obstetric complications are a well-known risk factor for schizophrenia and other psychotic disorders (Cannon *et al.*, 2002), in our cohort we had no access to reliable information on birth history.

Patients and controls had a similar sex and tobacco smoking status distribution. However, compared with the controls, the patients were significantly younger, less likely to be married, and had fewer years of education. Of the 47 patients included in the DAT study, 43 completed the GAF, 42 the PANSS, and 39 the CGI scales (Table A). Of the 36 patients included in the P300 study, 33 completed the PANSS and 25 completed the CGI-S (Table B).

Table A. Detailed Demographic Characteristics of the Participants in chapter 2 (DAT study)

	Schizophrenia (<i>n</i> = 47)	Controls (<i>n</i> = 112)	Statistics		
	Mean (SD)	Mean (SD)	<i>t</i> / χ^2	<i>df</i>	<i>p</i>
Age (years)	27.2 (8.7)	34.3 (12.0)	-4.19	117.7	<0.001
range	17.0-52.7	18.9-58.8			
Sex (male/female)	26/21	59/53	0.09	1	0.761
Smoking status (yes/no)	5/42	19/93	1.03	1	0.300
Marital status(M/S) [§]	5/42	42/70	11.47	1	0.001
Years of education	12.6 (3.2)	14.2 (3.5)	-2.62	154	0.010
range	5-21	0-22			
Age of onset	23.5 (7.4)	NA	NA	NA	NA
range	14-44				
Family history of psychiatric illness (%)	14/33	NA	NA	NA	NA
Alcohol use (Y/N)	1/44	5/107	0.44	1	0.51
Suicide attempt (Y/N)	3/44	NA	NA	NA	NA
PANSS (<i>n</i> =42)					
Positive	22.0 (6.2) 7-34	NA	NA	NA	NA
Negative	20.0 (7.2) 8-42	NA	NA	NA	NA
General Psychopathology	37.2 (11.6) 16-62	NA	NA	NA	NA

Total PANSS	79.3 (21.6) 32-138	NA	NA	NA	NA
CGI- Severity of Illness (<i>n</i> =39)	4.7 (1.0) 2-6	NA	NA	NA	NA
GAF (<i>n</i> =43)	41.0 (15.0) 5-75	NA	NA	NA	NA

[§]: M, includes married and living with a partner. S, includes single, divorced, married but separated.

PANSS: Positive and negative syndrome scale

CGI: Clinical global impression

GAF: Global assessment of functioning

Table B. Detailed Demographic Characteristics of the Participants in chapter 3 (P300 study)

	Schizophrenia (<i>n</i> = 36)	Controls (<i>n</i> = 138)	Statistics		
	Mean (SD)	Mean (SD)	<i>t</i> / χ^2	<i>df</i>	<i>p</i>
Age (years)	24.7 (5.8)	30.6 (10.0)	4.56	95.42	<0.001
range	18-39	19-58			
Sex (male/female)	21/15	74/64	0.26	1	0.61
Smoking (yes/no)	3/29	25/109	1.59	1	0.21
Marital Status (M/S) ^a	3/33	42/95	7.38	1	0.007
Years of education	13.3 (2.9)	15.0 (2.7)	3.32	171	0.001
range	5-21	6-24			
Age of onset	23.3 (5.8)	NA	NA	NA	NA
range	15-39				
Family history of psychiatric illness (%)	9/27	NA	NA	NA	NA
Alcohol use (Y/N)	2/34	6/130	0.08	1	0.77
Suicide attempt (Y/N)	1/32	NA	NA	NA	NA
PANSS (<i>n</i> =33)					
Positive	21.4 (5.1) 8-30	NA	NA	NA	NA
Negative	18.9 (7.4) 8-43	NA	NA	NA	NA
General Psychopathology	36.7 (13.5) 16-78	NA	NA	NA	NA
Total PANSS	76.9 (23.0)	NA	NA	NA	NA

	32-143				
CGI- Severity of Illness (<i>n</i> =25)	4.7 (1.1) 2-6	NA	NA	NA	NA
GAF (<i>n</i> =33)	45.5 (15.0) 5-75	NA	NA	NA	NA

^a: M, includes married and living with the partner. S, includes single, divorced, married but separated.

PANSS: Positive and negative syndrome scale

CGI: Clinical global impression

GAF: Global assessment of functioning

APPENDIX 2

THE INFLUENCE OF AGE ON RECEPTOR BINDING AND P300 AND A COMPARISON BETWEEN THE TWO

The main aim of my study was to assess the differences between patients and controls in the specific striatal [^{99m}Tc]-TRODAT-1 binding, considering both the left and right striatum measures. Mixed modeling was used to compare [^{99m}Tc]-TRODAT-1 binding between the 2 clinical groups, and to allow for possible group differences between left and right sites, we tested if a group by laterality interaction was evident.

On the basis of previous literature, we considered that age, sex, and tobacco smoking are potential confounders and therefore included these as covariates in the analysis (Kuikka *et al.*, 1997; Mozley *et al.*, 1999; Newberg *et al.*, 2007). The models included subject-varying intercepts to acknowledge correlation between the 2 repeated measures per participant.

The second aim of this study was to evaluate the effects of aging, sex, and tobacco smoking on the specific striatal [^{99m}Tc]-TRODAT-1 binding, and we present the regression coefficients obtained from the model described above. My third and final objective was to use the data to assess if any group effect varies with age or sex. Thus, we expanded the above model to test an interaction of group by age or group by sex, respectively.

Here I list the detailed results of statistical analyses using SPSS version16 (SPSS Inc., Chicago, IL, USA). Key results are highlighted in yellow.

Additional statistical analyses on TRODAT binding

The group by laterality interaction was not significant and was therefore dropped from our model ($F = 2.02$, $df = 1, 157$; $P = 0.16$).

After controlling for age, sex, and tobacco smoking, the mean specific striatal binding showed a trend for a difference between patients and controls, where patients had a reduction in their TRODAT binding (estimated difference between controls and patients = 0.071; 95% CI 0.01, 0.15; $F = 3.19$, $df = 1, 154$; $P = 0.08$). Please see Tables A & B.

In the same model, there was a significant effect of sex, whereby females had a higher ratio of the specific striatal binding than males (estimated difference between males and females = 0.08; 95% CI 0.15, 0.002; $F = 4.11$, $df = 1, 154$; $P = 0.045$). Please see Table C.

Tobacco smoking did not have a significant influence on TRODAT binding (estimated difference between smokers and nonsmokers = 0.06; 95% CI 0.17, 0.04; $F = 1.39$, $df = 1, 154$; $P = 0.24$), please see Table C. Finally, there was a highly significant effect of age, whereby TRODAT binding declined with advancing age (estimated change per decade of age = 0.1; 95% CI 1.2, 0.6; $F = 31.87$, $df = 1, 154$; $P < .001$). Please see Table C.

Table A

Coefficients^a

Model		Unstandardized Coefficients	
		B	Std. Error
1	(Constant)	2.562	.064
	age	-.009	.002
	sex	-.078	.038
	tobacco use	-.063	.053
	group	-.071	.040

Table B**Coefficients ^a**

Model		Standardized Coefficients	t	Sig.	95.0% Confidence Interval for B	
		Beta			Lower Bound	Upper Bound
1	(Constant)		39.891	.000	2.436	2.689
	age	-.432	-5.644	.000	-0.0122	-0.0059
	sex	-.161	-2.023	.045	-0.1538	-0.0018
	tobacco use	-.094	-1.181	.239	-0.1678	0.0422
	group	-.135	-1.784	.076	-0.1503	0.0077

Table C**Tests of Between-Subjects Effects**

Dependent Variable: TRODAT binding availability

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1.758 ^a	4	.440	9.118	<.001
Intercept	91.055	1	91.055	1888.722	<.001
age	1.536	1	1.536	31.855	<.001
group	.153	1	.153	3.182	.076
sex	.197	1	.197	4.092	.045
tobacco use	.067	1	.067	1.394	.239
Error	7.424	154	.048		
Total	778.325	159			
Corrected Total	9.183	158			

a. R Squared = .191 (Adjusted R Squared = .170)

Of note, there was no significant interaction between age and group ($F = 0.37$, $df = 1, 153$; $P = .55$), please see Table D, or between group and sex ($F = 0.20$, $df = 1, 153$; $P = .66$), please see Table E, indicating that the age decline in TRODAT was similar in patients and controls as well as in both sexes. Finally, again no significant interaction between age and sex was found on the DAT binding, please see Table F.

Table D**Tests of Between-Subjects Effects**

Dependent Variable: TRODAT binding availability

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1.776 ^a	5	.355	7.338	<.001
Intercept	64.139	1	64.139	1324.956	<.001
age	.763	1	.763	15.762	<.001
group	.063	1	.063	1.297	.257
sex	.203	1	.203	4.200	.042
tobacco use	.076	1	.076	1.565	.213
group * age	.018	1	.018	.368	.545
Error	7.407	153	.048		
Total	778.325	159			
Corrected Total	9.183	158			

a. R Squared = .193 (Adjusted R Squared = .167)

Table E**Tests of Between-Subjects Effects**

Dependent Variable: TRODAT binding availability

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1.768 ^a	5	.354	7.296	<.001
Intercept	89.648	1	89.648	1849.823	<.001
age	1.539	1	1.539	31.759	<.001
group	.160	1	.160	3.310	.071
sex	.148	1	.148	3.056	.082
tobacco use	.062	1	.062	1.284	.259
group * sex	.010	1	.010	.197	.658
Error	7.415	153	.048		
Total	778.325	159			
Corrected Total	9.183	158			

a. R Squared = .193 (Adjusted R Squared = .166)

Table F**Tests of Between-Subjects Effects**

Dependent Variable: TRODAT binding availability

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1.773 ^a	5	.355	7.323	<.001
Intercept	90.725	1	90.725	1873.392	<.001
age	1.550	1	1.550	32.011	<.001
group	.145	1	.145	3.000	.085
sex	.002	1	.002	.045	.832
Tobacco use	.065	1	.065	1.343	.248
sex * age	.015	1	.015	.307	.580
Error	7.409	153	.048		
Total	778.325	159			
Corrected Total	9.183	158			

a. R Squared = .193 (Adjusted R Squared = .167)

Additional statistical analyses on P300 amplitude and P300 latency performance

Based on equivalent statistical analyses used in the DAT binding section above, no significant interactions between age and sex were found on the P300 amplitude and latency. In Appendix 4 below, we have undertaken additional analyses examining the effects of age and sex on P300 performance. This includes a re-analysis of a sub-sample of patients and controls that were well matched demographically. Finally further analyses are presented on the full patient and control sample that compare the P300 models with and without adjustment by sex and age (Appendix 4).

In conclusion and consistently with the literature, TRODAT binding declined with age in my sample. This effect was observed both in patients and in controls, and in both sexes. Again in my study we found the expected ageing effects on P300 performance, which were in line with previous research.

APPENDIX 3

METHODOLOGICAL CONSIDERATIONS – INCLUSION OF CORRECT TRIALS IN THE AVERAGING OF EEG SIGNALS

The P300 event related potential is typically measured from an average of multiple single trials. The amplitude reductions described amongst patients with schizophrenia are observable even after trials with no visible P300 waveform are excluded and when trials are latency adjusted (Ford *et al.*, 1994a).

In the extensive literature investigating the P300 waveform in schizophrenia, the classical approach has been to average trials containing standard and target stimuli separately and to only include in the averages those trials eliciting correct responses from the participant (no response to standard tones and button-press or other response to targets) (Ford *et al.*, 1994a). Thus, in order to measure the P300 amplitude and latency the majority of studies have averaged only the artefact-free, correctly identified target trials (Ford *et al.*, 1994a; Frangou *et al.*, 1997; Bramon *et al.*, 2005; Bramon *et al.*, 2008). The paradigm we selected for my sample was a two-tone oddball and in all respects was comparable to these studies mentioned. Accordingly, we decided to undertake a similar analysis based on averaging only the correctly identified target trials. Furthermore, it is the correct identification of the target tones that is thought to generate the P300 waveform (Ford *et al.*, 1994a; Falkenstein *et al.*, 1997). The rationale for taking this approach is to maximise data quality and facilitate interpretation. If EEG epochs generated during missed targets were to be averaged together with correctly identified targets, a number of confounding effects on the underlying cognitive process might arise and interpretation would be more difficult (Ford *et al.*, 1994a; Frangou *et al.*, 1997).

Nevertheless, the design of my oddball experiment, just as was the case in many several preceding studies (Frangou *et al.*, 1997; Bramon *et al.*, 2005; Bramon *et al.*, 2008), involved the use of an easy task. The target and standard tones had frequencies of 2000 and 1000 Hz respectively. This large difference in pitch made it very easy for all study participants including patients with

schizophrenia to identify the targets. Given the easy nature of the oddball task in my study, the number of missed targets was very small and their inclusion would have been unlikely to influence the P300 amplitude and latency calculations in any event.

Table A gives details on the performance to my oddball task at a behavioural level. The accuracy of patients and controls is similar and very high. The patients and controls were able to correctly identify 99% of targets. Thus, including the targets that participants failed to correctly respond to, would result in adding only another 2 trials per subject on average and this would be unlikely to have a significant influence on the P300 waveforms we obtained. P300 oddball tasks have traditionally been designed to be relatively easy in order to ensure a sufficient number of correctly identified trials can be included in the averages for most participants thus maximising data quality.

Table A: Group comparisons on behavioural accuracy to the oddball task

	Controls	Patients	Statistics (independent samples T test)
Sample size	N=135	N=35	
% of total correct responses Mean (\pm SD)	99.2 (2.1)	98.6 (4.2)	p=0.24
Sample size	N=129	N=29	
% of correctly identified targets Mean (\pm SD)	96.1 (11.7)	95.4 (11.9)	p=0.71
% of correctly identified standards Mean (\pm SD)	99.9 (0.4)	99.7 (0.9)	p=0.22

This experiment consisted of 200 tones (80% standards and 20% targets). The subject was instructed to respond to the targets only. Using independent samples

T test, there was no significant difference in accuracy of behavioural response to the oddball task between the two groups.

METHODOLOGICAL CONSIDERATIONS – JUSTIFICATION OF SITES SELECTED FOR INVESTIGATION

The majority of papers in the schizophrenia P300 literature have investigated midline electrodes (Frangou *et al.*, 1997; Jeon & Polich, 2001; Bramon *et al.*, 2004a; Bramon *et al.*, 2005; Polich, 2007; Bramon *et al.*, 2008). The P300 signal is strongest and most reliably measured in central parietal areas. This is the reason most research in the field reports on the PZ and the CZ sites. The scalp distribution of the P300 signal typically shows amplitude increases from frontal to parietal electrode sites (Johnson, 1993).

However, as in other auditory tasks, temporal areas are also likely to be relevant. Indeed, P300 differences between patients with schizophrenia and controls have also been described in temporal areas bilaterally (Jeon & Polich, 2001; Kim *et al.*, 2014). Asymmetries have been described in schizophrenia in laterality comparisons (McCarley *et al.*, 1993; McCarley *et al.*, 2002).

In my study, the EEG was acquired over 32 electrodes using the 10/20 international system (Jasper, 1958). However, for the P300 experiment only three midline electrodes FZ, CZ and PZ were analysed. Although it would certainly have been possible to analyse many alternative sites, given that our sample included 36 patients and 138 controls, some form of data reduction was needed. I considered the option of undertaking a factor analysis of all 32 sites in order to extract 2 or 3 key components from each individual for subsequent analysis. However this approach is rarely used in the P300 schizophrenia literature and this would make putting my findings into context potentially difficult. In the end, I opted for focusing on the three midline electrodes because this approach has been most commonly used in the P300 schizophrenia literature and would allow a direct comparison of my data with extensive previous research.

APPENDIX 4

THE DEMOGRAPHIC MATCHING OF PATIENTS AND CONTROLS IN THIS STUDY

In my study, the patients and controls had significant differences in age distribution. The sex distribution was however balanced with no significant group differences (table 1 of Chapter 3). Indeed, the age range of the control and the patient groups was respectively 19-58 and 18-39 years, and the mean age was significantly younger amongst patients compared to controls. Even though all my analyses are adjusted for age, the inclusion of older controls could have affected my results. A well-established effect of ageing is to increase the latency and to a lesser extent to reduce the amplitude of the P300 (Polich *et al.*, 1985; Jeon & Polich, 2001). Therefore, it is conceivable that with age matched patients and controls I might have found P300 amplitude and latency group differences as previous studies have done (Wang *et al.*, 2003). In my thesis I opted to include all my study participants (36 patients and 138 controls), despite the age group imbalances, in order to maximise statistical power.

Nevertheless, I have now conducted further analyses with a subgroup of randomly-selected 30 patients and 30 controls individually matched by age and sex (Table A). No difference between groups was found in P300 amplitude or latency in the matched sub-sample (Table A and Table B). I acknowledge that by reducing the sample size, statistical power has been reduced.

Table A. Demographic characteristics of patients with schizophrenia ($n = 30$) and age and sex matched controls ($n = 30$)

	Patients	Controls	Statistical test		
	Mean (SD)	Mean (SD)	t/χ^2	df	p
Age (years)	25.70 (5.63)	25.73 (5.72)	-0.02	58	0.98
range	18-39	19-40			
Sex (male/female)	15/15	15/15	-	-	-
Smoking (yes/no)	2/24	6/24	1.72	1	0.19
Marital Status (M/S) ^a	3/27	4/26	0.16	1	0.69
Years of education	13.60 (3.04)	14.87 (2.50)	-1.76	58	0.08
range	5-21	9-22			
Striatal DAT availability ^b	2.21 (0.23)	2.34 (0.22)			
range	1.79-2.66 ($n=18$)	1.89-2.71 ($n=28$)	1.89	44	0.07
Duration of illness (months)	40.2 (72.3)	NA	NA	NA	NA
range	1.0-288.8				
PANSS ($n=28$)					
Positive	21.5 (5.4) 8-30	NA	NA	NA	NA
Negative	19.0 (7.7) 8-43	NA	NA	NA	NA
General Psychopathology	36.6 (13.9) 16-78	NA	NA	NA	NA
Total PANSS	77.1 (23.7) 32-143	NA	NA	NA	NA
CGI- severity of Illness ($n=21$)	4.7 (1.1) 2-6	NA	NA	NA	NA

^a: M, includes married and living with the partner. S, includes single, divorced, married but separated.

^b: The specific striatal [^{99m}Tc]-TRODAT-1 binding as the measure of dopamine transporter (DAT) availability was calculated via the mean count of regions of interest in the striatum divided by it in the occipital region (St/Oc).

PANSS: Positive and negative syndrome scale

CGI: Clinical global impression

Table B. Latency / amplitude of patients with schizophrenia ($n = 30$) and age and sex matched controls ($n = 30$)

	Parietal		Central		Frontal		Statistical analysis	
	Mean	(SD)	Mean	(SD)	Mean	(SD)	coefficient	p
Latency (ms)								
Schizophrenia	339.93	(31.07)	336.07	(33.44)	336.87	(33.93)	-5.70	0.48
Normal control	332.97	(31.77)	331.57	(31.94)	331.17	(29.80)		
Amplitude (μV)								
Schizophrenia	7.94	(3.88)	7.61	(3.91)	6.32	(3.37)	0.77	0.40
Normal control	9.10	(4.32)	7.99	(4.41)	7.07	(4.77)		

The findings prior to co-variation for age and sex and after co-variation for age and sex are shown below (Table C)

Table C: Latency/amplitude of patients with schizophrenia ($n=36$) & controls ($n=138$)

	Parietal		Central		Frontal		Statistical analysis					
							Controlling for age, sex, and electrode site			Controlling only for electrode site		
	Mean	(SD)	Mean	(SD)	Mean	(SD)	coefficient	t	p	coefficient	t	p
Latency (ms)												
Schizophrenia	338.9	(29.2)	335.0	(31.2)	335.7	(32.6)	-3.85	-0.65	0.52	-5.02	0.88	0.38
Normal control	334.7	(32.5)	331.2	(32.8)	328.7	(33.8)						
Amplitude (μ V)												
Schizophrenia	8.09	(4.20)	7.63	(4.00)	6.31	(3.32)	0.27	0.39	0.71	0.17	0.17	0.87
Normal control	8.00	(4.04)	7.32	(4.29)	7.08	(4.42)						

As per table C, prior to co-variation by age and sex there were no significant group differences between patients and controls in P300 amplitude or latency. These results are similar to the age and sex adjusted analyses included in the thesis.

APPENDIX 5

A RE-ANALYSIS OF SYMPTOM CLUSTERS USING A FIVE-FACTOR MODEL OF THE PANSS IN MY PATIENT SAMPLE

A five-factor model of the PANSS for the Chinese population

The Positive and Negative Syndrome Scale (PANSS) is widely used to assess the severity of symptoms among patients with schizophrenia. A total of 30 items were designed to assess the severity of three a priori major symptom dimensions: A positive syndrome (7 items), a negative syndrome (7 items), and general psychopathology (16 items) (Kay *et al.*, 1987). Subsequent studies have shown that the PANSS can measure five dimensions of symptomatology of schizophrenia. Wallwork *et al.* (2012) have built a consensus model by assigning only the consistent PANSS items to each of the five factors based on previously published models and advocate in favour of this five factor approach (Wallwork *et al.*, 2012). However, few studies have ever investigated the structure of the PANSS in a Chinese schizophrenia population. A Chinese version the five-factor model of PANSS has been proposed which includes a positive, negative, excitement, depression and cognitive factors (Jiang *et al.*, 2013). The items loaded on these factors were similar to the consensus items published in previous studies, except for PANSS items P2 conceptual disorganization, P5 grandiosity, N5 abstract thinking, and G11 poor attention. This discrepancy is thought to be due to the influence of culture on the clinical presentation of schizophrenia. Table A shows details of the validation study of the five-factor PANSS modelling for schizophrenia in the Chinese population (Jiang *et al.*, 2013).

Table A. Validated five-factor model of the PANSS for schizophrenia in the Chinese population

Factor loadings of the PANSS items and the number of models that have assigned the corresponding items to the corresponding factors in the 32 published models.

	Positive	N	Negative	N	Excitement	N	Depression	N	Cognitive	N
P1: Delusions	1.000	32		0		0		0		0
P2: Conceptual disorganization	<i>0.430</i>	<i>1</i>		0	<i>0.495</i>	0		2		27
P3: Hallucinations	0.576	28		0		1		2		1
P4: Hyperactivity		0		0	0.803	30		1		1
P5: Grandiosity		25		0	0.546	1		0		1
P6: Suspiciousness	0.844	22		0		2		2		0
P7: Hostility		0		0	0.839	32		0		0
N1: Blunted affect		0	<i>0.440</i>	<i>30</i>		0		0	<i>0.461</i>	0
N2: Emotional withdraw		0	0.876	31		0		0		0
N3: Poor rapport		0	0.932	31		1		1		0
N4: Social withdrawal		0	0.916	30		0		1		0
N5: Abstract thinking		0		1		0		0		30
N6: Spontaneity		0	0.908	31		0		0		1
N7: Stereotyped		4		1	0.702	3		0		14
G1: Somatic concern		5		0		0	0.443	17		0
G2: Anxiety		0		0		1	0.673	29		0
G3: Guilt feelings		0		1		0	0.731	29		0
G4: Tension		1		0		12	0.445	13		1
G5: Mannerisms		0		5	0.53	1		1		15
G6: Depression		0		0		0	0.659	30		0
G7: Motor retardation		0	0.557	28		0		0		1
G8: Uncooperativeness		0	<i>0.415</i>	<i>4</i>	<i>0.723</i>	<i>27</i>		0		0
G9: Unusual thought	0.591	26		0		0		0		5
G10: Disorientation		1		0		2		0	0.605	22
G11: Poor attention		1		3	0.616	0		1		26
G12: Lack of judgment/insight		13		1		2		2	0.629	4
G13: Disturbance of volition		1	0.537	9		0		1		9
G14: Poor impulse control		0		1	0.716	29		0		1
G15: Preoccupation		1		4	0.536	0		6		11
G16: Active social avoidance		1	0.597	19		2		4		1

N is the number of models out of the 32 reviewed models that have assigned the corresponding items to the corresponding factor. All displayed items load over 0.400 onto a single factor. Items in italics load onto more than one factors with loading of over 0.400. Items in bold are the chosen items loaded onto the corresponding factor in the final model.

This table shows the factor loadings of the PANSS items and the number of models that have assigned the corresponding items to the corresponding factors in the 32 published models as reviewed by Jiang et al, 2013.

The relationship between the five-factor PANSS and DAT availability in my sample of antipsychotic naïve patients with schizophrenia

I used the five-factor model of the PANSS, Chinese version from Jiang et al as the basis of my analyses in order to explore the associations between PANSS and DAT availability. The factor loadings of the PANSS items and the number of models that have assigned the corresponding items to the corresponding factors are showed in Table A. No significant association was found between striatal DAT availability and each of the five factors of the PANSS that we examined. These results are shown in Table B (Appendix 5).

Table B. Correlations between Striatal DAT availability and PANSS scores in my patient sample (n=42) using Jiang's five-factor model

		Positive	Negative	Excitement	Depression	Cognitive
PANSS						
Mean (SD)		12.51 (3.69)	11.06 (4.60)	5.65 (2.66)	4.06 (1.37)	2.98 (1.23)
Range		3.01-19.33	3.26-23.46	2.36-11.88	2.06-6.93	1.23-6.80
Striatal	ρ	0.13	0.05	0.22	0.23	0.13
DAT						
availability	p	0.41	0.78	0.16	0.14	0.40

DAT: Dopamine transporter

PANSS: Positive and negative syndrome scale

ρ : Spearman's Rho Correlation coefficients

p: significance value of correlation analyses

The relationship between the five-factor PANSS and P300 ERP in my sample of antipsychotic naïve patients with schizophrenia

The P300 is a positive-going wave with a scalp amplitude distribution in which it is largest in parietal sites (Pz) and smallest frontally (Fz), taking intermediate values centrally (Cz). In a new analysis of our patient data, the P300 amplitude was found to negatively correlate with the PANSS cognitive factor (disorientation, lack of judgement/insight) [mean (SD) = 3.15 (1.83), Pz, ρ = -0.44, p = 0.01] and with the PANSS positive factor (delusions, hallucinations, suspiciousness, unusual thought) [mean (SD) = 12.96 (4.07), Cz, ρ = -0.39, p = 0.02].

The P300 latency was found to positively correlate with the PANSS cognitive factor [mean(SD)= 3.15 (1.83), P_z , $\rho=0.48$, $p=0.01$], with the depression factor (anxiety, guilt feelings, depression) [mean(SD)= 4.70 (3.14), P_z , $\rho=0.40$, $p=0.02$], and finally with the excitement factor (hyperactivity, hostility, poor impulse control) [mean(SD)= 5.40 (2.27), P_z , $\rho=0.40$, $p=0.04$]. These analyses are fully reported in Table C.

Patients with schizophrenia have P300 amplitude reductions and latency prolongations compared to healthy controls (Frangou *et al.*, 1997; Coburn *et al.*, 1998; Ozgurdal *et al.*, 2008; Shin *et al.*, 2010; Jahshan *et al.*, 2013). Even though the number of available studies is much smaller, antipsychotic free patients with schizophrenia have also been shown to have significantly attenuated amplitude and delayed latency on oddball paradigms eliciting the P300 response (Coburn *et al.*, 1998; Bramon *et al.*, 2004b). My results on the P300 amplitude are in line with the findings by Higashima *et al.*, who reported that the score for the thought disorder factor of the PANSS correlated negatively with the amplitude of P300 in patients with schizophrenia (Higashima *et al.*, 1998). My results are further supported by Kirihaara *et al.*, who also reported that the auditory P300 amplitude showed a significant negative correlation with two different measures of severity of thought disorder (Johnston & Holzman, 1979; Marengo *et al.*, 1986). Thus the authors concluded that electrophysiological abnormalities of information processing may underlie the presence of thought disorder in schizophrenia (Kirihaara *et al.*, 2005).

Moreover, P300 amplitude has been also implicated in other studies as a clinical-state marker in schizophrenia by showing associations with clinical symptoms (Pfefferbaum *et al.*, 1989; Eikmeier *et al.*, 1992; Egan *et al.*, 1994; Maeda *et al.*, 1996; Turetsky *et al.*, 1998b; Ford *et al.*, 1999; Mathalon *et al.*, 2000a). It tracked Brief Psychiatric Rating Scale (BPRS) total scores and positive symptom scores over time, with decreasing amplitude during symptom exacerbations (Mathalon *et al.*, 2000a). Consistently with the above studies, in a study of males with recent onset psychosis, the P300 amplitude correlated with

symptom severity and could explain 66% of the patient's performance in total PANSS scores (Sumich *et al.*, 2006). Based on all these studies I would tentatively suggest that the P300 amplitude could be a physiological correlate of psychosis severity, especially reflecting degree of cognitive impairment and positive symptom severity.

Again in my sample, the P300 latency positively correlates with the depression, excitement, and cognitive factors of the PANSS. These findings are also in line with the studies of major depressive disorder that previously reported P300 latencies were significantly prolonged both in major depressive patients with and without psychotic features compared to controls. Furthermore, P300 amplitudes were significantly decreased only in the major depressive patients with psychotic features before treatment (Vandoolaeghe *et al.*, 1998; Karaaslan *et al.*, 2003).

According to the aforementioned results (Appendix 1 Tables A & B, Appendix 5 Tables B & C), the symptomatology of patients recruited in my studies was not severe and this is reflected in their PANSS scores. This may have contributed to our failure to identify overall P300 differences between patients and controls in our study. However, there were significant associations between some symptom clusters, for example positive symptoms, cognitive and excitement factors. This suggests that a more symptomatic sample may indeed have given rise to a distinctive pattern of results for the patient group. Please note, these additional analyses are exploratory and statistical significance levels have not been adjusted for multiple comparisons.

Table C. Correlations between P300 latency/amplitude and PANSS in my patient sample (n=33) using Jiang's five-factor model

		Positive	Negative	Excitement	Depression	Cognitive
PANSS						
Mean (SD)		12.96 (4.07)	10.43 (5.05)	5.40 (2.27)	4.70 (3.14)	3.15 (1.83)
Range		3.01-27.10	4.19-29.32	2.36-10.99	2.06-18.57	1.23-11.11
Latency (ms)						
Parietal	ρ	0.30	0.22	0.37	0.40	0.480
	p	0.09	0.23	0.035	0.020	0.005
Central	ρ	0.29	0.09	0.33	0.40	0.35
	p	0.11	0.60	0.06	0.023	0.047
Frontal	ρ	0.23	-0.01	0.24	0.34	0.23
	p	0.20	0.98	0.18	0.053	0.20
Amplitude (μ V)						
Parietal	ρ	-0.32	-0.23	-0.05	-0.02	-0.44
	p	0.07	0.19	0.78	0.89	0.011
Central	ρ	-0.39	-0.22	-0.25	-0.08	-0.50
	p	0.024	0.22	0.17	0.65	0.003
Frontal	ρ	-0.33	-0.20	-0.35	-0.08	-0.51
	p	0.06	0.28	0.046	0.64	0.003

PANSS: Positive and negative syndrome scale

ρ : Spearman's Rho Correlation coefficients

p: significance value of correlation analyses

Examiners' Report:

We had a thorough look through the Reply and the revised Thesis. Dr Chen has comprehensively addressed our points.



Signed: _____ (K. Ebmeier)

And:  (A. SUMICH)

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